**Compilation of 1989 Annual Reports
of the Navy ELF Communications System
Ecological Monitoring Program**

Volume 2 of 3 Volumes:
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This is the eighth compilation of annual reports for the Navy's ELF Communications System Ecological Monitoring Program. The reports document the progress of eight studies performed during 1989 near the Naval Radio Transmitting Facility -- Republic, Michigan. The purpose of the monitoring is to determine whether electromagnetic fields produced by the ELF Communications System will affect resident biota or their ecological relationships. ⟶ next page

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F. Small Mammals and Nesting Birds:
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FOREWORD

During 1989, the U.S. Department of the Navy continued to conduct a long-term program to monitor for possible effects to resident biota and their ecological relationships from operation of the Navy's Extremely Low Frequency (ELF) Communications System. These studies were funded by the Space and Naval Warfare Systems Command (SPAWAR) through a contract to IIT Research Institute (IITRI). IITRI provided engineering support and overall program management for the ecological studies. Monitoring projects were funded as subcontract agreements between IITRI and university investigators.

The compiled reports document the technical progress and findings of monitoring projects performed near the Naval Radio Transmitting Facility—Republic, Michigan during 1989. As in the past, each report has been reviewed by four or more scientific peers, and investigators have considered and addressed peer critiques prior to providing a final document for this compilation. The reports are presented without further change or editing by SPAWAR or IITRI.

Data collection for studies at the Naval Radio Transmitting Facility—Clam Lake, Wisconsin were completed during 1989, as scheduled. The results and conclusions of studies of bird species and communities are included in this compilation; a final summary report based on data collected over the entire term of the project should be available by the end of 1990. Final summary reports for the other Wisconsin studies (wetland flora and slime molds) are available from the National Technical Information Service (NTIS).

Past compilations, executive summaries, and engineering reports are also available from NTIS. A listing of all documents prepared since the inception of the program in 1982 follows the index of 1989 annual reports.

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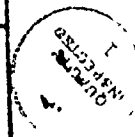
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ECOLOGICAL MONITORING PROGRAM

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Final Reports

1. Guntenspergen, G.; Keough, J.; Stearns, F.; Wilkum, D. ELF Communications System Ecological Monitoring Program: Wetland Studies--Final Report. IIT Research Institute, Technical Report E06620-2, 1989. 162 pp. plus appendixes.

2. Goodman, E.; Greenebaum, B. ELF Communications System Ecological Monitoring Program: Slime Mold Studies--Final Report. IIT Research Institute, Technical Report E06620-3, 1990. 43 pp. plus appendixes.

Compilations

3. Compilation of 1988 Annual Reports of the Navy ELF Communications System Ecological Monitoring Program. IIT Research Institute, Technical Report E06595-6, 1989. Vol. 1, 572 pp.; Vol. 2, 351 pp.; Vol. 3, 449 pp.

4. Compilation of 1987 Annual Reports of the Navy ELF Communications System Ecological Monitoring Program. IIT Research Institute, Technical Report E06595-2, 1988. Vol. 1, 706 pp.; Vol. 2, 385 pp.; Vol. 3, 491 pp.

5. Compilation of 1986 Annual Reports of the Navy ELF Communications System Ecological Monitoring Program. IIT Research Institute, Technical Report E06549-38, 1987. Vol. 1, 445 pp.; Vol. 2, 343 pp.; Vol. 3, 418 pp.

6. Compilation of 1985 Annual Reports of the Navy ELF Communications System Ecological Monitoring Program. IIT Research Institute, Technical Report E06549-26, 1986. Vol. 1, 472 pp.; Vol. 2, 402 pp.; Vol. 3, 410 pp.

7. Compilation of 1984 Annual Reports of the Navy ELF Communications System Ecological Monitoring Program. IIT Research Institute, Technical Report E06549-17, 1985. Vol. 1, 528 pp.; Vol. 2, 578 pp.

8. Compilation of 1983 Annual Reports of the Navy ELF Communications System Ecological Monitoring Program. IIT Research Institute, Technical Report E06549-8, 1984. Vol. 1, 540 pp.; Vol. 2, 567 pp.

9. Compilation of 1982 Annual Reports of the Navy ELF Communications System Ecological Monitoring Program. IIT Research Institute, Technical Report E06516-5, 1983, 402 pp.

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10. Haradem, D. P.; Gauger, J. R.; Zapotosky, J. E. ELF Communications System Ecological Monitoring Program: Electromagnetic Field Measurements and Engineering Support—1988. IIT Research Institute, Technical Report E06595-5, 1989, 69 pp. plus appendixes.

11. Haradem, D. P.; Gauger, J. R.; Zapotosky, J. E. ELF Communications System Ecological Monitoring Program: Electromagnetic Field Measurements and Engineering Support—1987. IIT Research Institute, Technical Report E06595-1, 1988, 54 pp. plus appendixes.

12. Haradem, D. P.; Gauger, J. R.; Zapotosky, J. E. ELF Communications System Ecological Monitoring Program: Electromagnetic Field Measurements and Engineering Support—1986. IIT Research Institute, Technical Report E06549-37, 1987, 52 pp. plus appendixes.

13. Brosh, R. M.; Gauger, J. R.; Zapotosky, J. E. ELF Communications System Ecological Monitoring Program: Electromagnetic Field Measurements and Engineering Support—1985. IIT Research Institute, Technical Report E06549-24, 1986, 48 pp. plus appendixes.

14. Brosh, R. M.; Gauger, J. R.; Zapotosky, J. E. ELF Communications System Ecological Monitoring Program: Measurement of ELF Electromagnetic Fields for Site Selection and Characterization—1984. IIT Research Institute, Technical Report E06549-14, 1985, 37 pp. plus appendixes.

15. Enk, J. O.; Gauger, J. R. ELF Communications System Ecological Monitoring Program: Measurement of ELF Electromagnetic Fields for Site Selection and Characterization—1983. IIT Research Institute, Technical Report E06549-10, 1985, 19 pp. plus appendixes.

Program Summaries

16. Zapotosky, J. E. Extremely Low Frequency (ELF) Communications System Ecological Monitoring Program: Summary of 1988 Progress. IIT Research Institute, Technical Report E06620-1, 1989, 74 pp. plus appendixes.

17. Zapotosky, J. E. Extremely Low Frequency (ELF) Communications System Ecological Monitoring Program: Summary of 1987 Progress. IIT Research Institute, Technical Report E06595-3, 1989, 64 pp. plus appendixes.

18. Zapotosky, J. E. Extremely Low Frequency (ELF) Communications System Ecological Monitoring Program: Summary of 1986 Progress. IIT Research Institute, Technical Report E06549-39, 1987, 63 pp. plus appendixes.

19. Zapotosky, J. E. Extremely Low Frequency (ELF) Communications System Ecological Monitoring Program: Summary of 1985 Progress. IIT Research Institute, Technical Report E06549-27, 1986, 54 pp. plus appendixes.

20. Zapotosky, J. E. Extremely Low Frequency (ELF) Communications System Ecological Monitoring Program: Summary of 1984 Progress. IIT Research Institute, Technical Report E06549-18, 1985, 54 pp. plus appendixes.

21. Zapotosky, J. E.; Abromavage, M. M.; Enk, J. O. Extremely Low Frequency (ELF) Communications System Ecological Monitoring Program: Summary of 1983 Progress. IIT Research Institute, Technical Report E06549-9, 1984, 49 pp. plus appendixes.

22. Zapotosky, J. E.; Abromavage, M. M. Extremely Low Frequency (ELF) Communications System Ecological Monitoring Program: Plan and Summary of 1982 Progress. IIT Research Institute, Technical Report E06516-6, 1983, 77 pp. plus appendixes.

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iii.

3. Table of Contents:

4. Abstract:

As was the case for the three prior years (i.e. 1986, 1987 and 1988), the 1989 growing season was a drought year. For the fourth growing season again growth was suppressed. This is in marked contrast with the 1984 and 85 growing seasons, in which abundant rainfall took place and the population increase in soil amoebae during the growing season was far greater than the drought years.

During the 1989 growing season the ELF antenna was operated intermittently. Assuming the antenna is fully operational for the 1990 season, this will provide 2 years of ELF exposure (i.e. 1990 and 1991) for the biological systems to react to the radiation. I think a third year of data would provide a better data base to judge whether there are biological effects created by ELF electromagnetic radiation.

The antenna, ground and control sites used in previous years were continued. The sites have been characterized by IITRI personnel so that all sites have a similar 60 cycle electromagnetic background while the control site is essentially devoid of ELF radiation from the antenna. I have been monitoring various physical and chemical properties of the sites as well as their biological characteristics.

At all sites, population size fluctuations were observed, as was the case in previous years. Of course the fluctuations were not as dramatic for the 4 drought seasons, as they were in 1984 and 1985. Techniques for measuring growth rates of amoebae in soil submerged cultures, developed over previous seasons, was perfected in the 1989 season.

5. Summary:

Plot selection and characterization: soil chemistry was performed on all sites in 1989, as was done in previous years. As in past years, differences in soil chemistry were noted between sites although these were within the same order of magnitude.

Species and strain characterization: In previous growth seasons, _Acanthamoeba polyphaga_ was used to test for strain heterogeneity within and between the sites. Isoenzyme analysis was used to detect strain differences. No differences were found between sites. Budget constraints forced the elimination of this aspect of the study. As noted below, isozyme analyses were used to detect possible changes in amoebae grown in the presence of the electric currents generated by the ELF antenna.

Population size: the fluctuation in population of amoebae during the growing season was determined as in past years. No differences were noted in total population size between study sites for a given horizon and sampling date except for the July count. As in past years differences were noted in the number of dormant cysts between sites. Again for 1989, a drought year, population sizes did not reach those observed in normal rainfall years (e.g. 1984, 1985).

Growth and feeding activity: to test growth rate of amoebae, soil submersible culture vessels were designed. Although the antenna was not fully operational in the 1989 growing season, it was used for sufficient time to do growth and isozyme experiments. Limited experiments did not reveal differences in growth rate or isoenzyme heterogeneity between sites.

Ambient monitoring: soil temperature and moisture were monitored continuously during the field season, as was done in previous years. The moisture content of soil in the Spring of 1986, 1987, 1988 and 1989 was lower than normal rainfall years. This and total rainfall correlated to small populations of amoebae in the soil during the drought years. Soil temperature over the growing season was the same between study sites. Temperature changes from Spring to Fall for 1989 ranged from approximately 10 to 19° C over the growing season.

6. Progress report:

OBJECTIVES: The project objective is to determine possible effects of ELF radiation on amoebae in soil. The sites chosen for this study are adjacent to the Michigan ELF transmitter facility.

For the 1989 field season, as was true for the previous seasons, the primary objective was to determine whether the control, antenna and ground wire sites were biologically similar in regards to soil amoebae. In addition a base line was accumulated for comparison with future data. Once the antenna is fully operational for a time span sufficient to expect possible effects, this background data will be especially useful.

WORK PLAN ELEMENTS:

#0. Plot selection and characterization.

Synopsis: site selection is complete. Statistical analysis of soil chemistry shows some variability between sites, as was the case for data from 1986, 1987 and 1988. This may be due to the prolonged drought that has continued from 1986. Prior to the drought (e.g 1985) differences were not observed between sites. Likewise this could be due to differences in the variability of data between years.

#1. Species and strain characterization.

Synopsis: using morphological and physiological markers, identify
species and strains of soil amoebae from the study areas so that
possible changes in the population due to ELF can be detected.
For budgetary reasons this portion of the work plan was deleted
for the 1989 season. The allozyme methods developed for genetic
analyses and a clonal isolate of A. polyphaga from previous years
was used in Work Plan #3.

#2. Population size and activity.

Synopsis: determine population size of amoebae in soil and the
ratio of vegetative to dormant amoebae over the growing season.
This is a productivity measure which could be affected by ELF
radiation. It could also be a reflection of changes in the
microbial food organisms due to ELF radiation. Direct ELF
effects on amoeba growth vs. indirect effects on food organisms
can be distinguished in two ways: growth of amoebae in culture
vessels exposed to ELF radiation (see #3 below) and a new
parameter described below, to be done in the 1990 season, i.e.
directs counts of bacteria in soil

Specifics: an established soil dilution counting procedure is
used (Singh, 1946 as modified by Darbyshire et al., 1974). In
order to count vegetative amoebae and cysts, samples are first
divided in half, one-half is used to count total cysts and
vegetative amoebae while the other half is treated to kill

amoebae so that only cysts are counted. Differential counts are
used to calculate by subtraction the total vegetative amoeba
count. In the 1983 season I found that 8 random samples,
subdivided into organic and mineral horizons (ie. 8 samples per
horizon), provided statistically significant data;

I will repeat this from the 1983 report: ten samples were
counted from each horizon at the three sites on two dates; the
results indicated a coefficient of variation that was less than
10% of the mean for a given horizon and date. From a 90% power
curve, significant differences could be detected at 1.4 X std.
dev. for a sample size of 10 and 1.5 to 1.6 X std. dev. for a
sample size of 8. Thus sample sizes of 8 and 10 were almost
equally powerful so that 8 random samples were taken from each
horizon at the three sample sites on a sampling date.

One-way analysis of variance was used to detect differences in
total amoeba and cyst count between control, antenna and ground
sites for each horizon in 1989. I was advised by Professor John
Gill at MSU, a statistician who works with biological problems,
that log transformed data should be used for statistical
analyses. Since the microbial population doubles over time, log
transformed data more closely reflects biological events. Table
4B gives the error (i.e. among) degrees of freedom as 21. Direct
counts of amoebae in soil, as is done with freshwater organisms
(e.g. Wright & Coffin, 1984) is not possible. Microbes adhere to
soil and sonication of a soil slurry to release them might make
quantitative recovery of some organisms by subsequent density
flotation possible, but amoebae would be destroyed.

In the 1990 season estimates of bacterial population sizes
will be estimated (Hanssen et al. 1974; Hobbie et al., 1977) to
see if there is a correlation to fluctuations in protozoan

populations.

#3. Growth and feeding activity.

Synopsis: determine the _in_ _situ_ growth and feeding activity of amoebae in soil submersible culture vessels. This will provide data on growth rate, feeding activity and mean generation time (i.e. the cell cycle between nuclear mitoses).

Rationale: the approach utilizes a known amoeba species previously isolated from the study site, Acanthamoeba polyphaga and characterized as part of the isoenzyme study. Direct counts of amoebae are made with a microscope to determine increase in number of organisms and nuclei over time. A log transform of these data provides a straight line plot which can be quantified by regression analysis. Statistically significant differences between slopes can be detected with confidence limits of the line, a version of the t-test. This approach will be used to determine growth rate and thus mean generation time. Mean generation time is comparable to the cell cycle measurement of time between mitoses of Physarum. Isozyme analyses are done before and after serial growth in the culture vessels subjected to ELF-induced electromagnetic effects, to detect possible genetic changes in the exposed amoebae. We had previously developed this technique (e.g. Jacobson & Band, 1987) for screening clonal isolates of A. polyphaga from soil to look for genetic effects, using Nei's (Nei, 1972) statistical methods for comparing data between sites. In view of the report by Goodman

et al. (1987), where changes in gene action were observed as a consequence of electromagnetic radiation, I hope to use a similar approach in the 1990 season. That is, amoebae grown in the presence of the electromagnetic current for the season will be analyzed with 2D-gel electrophoresis to see if changes in gene action can be detected.

Culture chambers, containing electrodes to use in conjunction with ELF induced soil currents, were designed with the help of IITRI personnel. To measure growth rate of amoebae directly in soil would be ideal, but the techniques to do this are inefficient, labor intensive and not as accurate as direct counts of amoebae (i.e. soil dilution counts similar to those used to measure the number of amoebae present in soil). Further, uncontrolled interactions with other soil organisms could affect amoeba growth. Soil water is a saline suitable for amoeba growth, but it does not exist as a continuous aqueous phase in soil. Therefore soil exhibits a higher electrical resistance than would be the case for soil water alone over a comparable distance, which is also the case for culture vessels, in which the saline is a continuous phase between the electrodes. Therefore two different culture vessel configurations are used, one to mimic the voltage induced in soil by the ELF radiation (with a greater current, since the resistance in saline is less than a comparable distance in soil) and the other to mimic soil current (with a smaller voltage than observed in soil). In previous seasons, it was established that chambers buried at research sites yielded growth rates that were not statistically

different. Since 1985, IITRI personnel have cooperated in the design and construction of electrical components used in the soil growth experiments. The recent design includes continuous recording of soil voltages throughout the season as well as providing the electrical connections to the soil submersible culture vessels.

#4. Ambient monitoring.

Synopsis: soil temperature and moisture are monitored. Both measures are useful for general trends but fail to correlate to changes in amoeba populations. The 4-year drought (i.e. 1986 to 1989) had a dramatic effect on soil amoeba population size although this was better reflected in annual precipitation patterns than in soil moisture. Soil temperature changes little over a growing season.

#5. Data analysis.

Synopsis: statistical analyses mentioned earlier are summarized here. For amoeba counts in soil, by soil dilution procedures, a one-way analysis of variance with 8 replicates per cell was adequate. One-way analysis of variance was used for soil counts (Table 4B) and soil moisture (Table 5) because it is not possible to compare accurately soil horizons or sampling dates. Soil horizons differ markedly in their densities. Bulk density of the organic and mineral horizons were presented in the 1983 annual

report; the ratio of mineral to organic soil was 2.9. When this ratio is used to compare soil counts of the organic and mineral horizons some data fits this ratio but in most cases only a tendency can be observed. Thus soil counts corrected for bulk density differences between organic and mineral horizons will tend to be closer in count so that the bulk density data does indicate that mineral horizon population sizes are not too different from population sizes in the overlying organic horizon. Moisture and counts differ between sampling dates. Growth measurements in culture chambers were analyzed with regression lines, comparing slopes with confidence intervals (i.e. a t-test). Other statistical comparisons (e.g. soil chemistry, soil pH, etc.) are done by analysis of variance. For isoenzyme determinations, comparisons between isolates are done by the method of Nei (1972).

SCHEDULE OF WORK ELEMENTS (Nov. 1 to ct. 31 each year)

	MONTH											
Element	1	2	3	4	5	6	7	8	9	10	11	12
0						X	X	X				
* 1												
2						X	X	X	X	X	X	
3	X	X	X	X	X	X	X	X	X	X	X	
4						X	X	X	X	X	X	
5	X	X	X	X	X							
Reports	X	X	X	X	X	X	X	X	X	X	X	X

* Omitted in the 1989 season.

EXPERIMENTAL

Methods and results will be presented in reference to the
Work Plan, given above.

#0. Plot selection and characterization. Site selection is
now complete.

Table 1 shows the chemical properties of the organic and
mineral horizons for the control, antenna and ground wire sites,
with replicates. As in past seasons, differences exist between
sites. This might be attributable to the drought which has
extended over four years, 1986, 1987, 1988 and 1989. The
chemical content of soil in 1989 was similar to 1988. As noted
in the 1985 report, in view of the wide fluctuation in population
size of amoebae seen throughout a given growing season, it would
be of interest to see if this is reflected in soil ammonium
levels. In consultation with Dr. J. Tiedje of the Department of
Crop and Soil Sciences, it was determined that the rapid passage
of ammonia through the ecosystem would make this impractical.
Thus soil ammonium has not been determined. Table 2 demonstrates
some significant differences between sites and sampling dates
although values shown in Table 1 are consistent between horizons.
Table 3 demonstrates the slightly acidic nature of the soil in a
northern hardwood forest, with significant differences between
sites and horizons for one sampling date (i.e. June 9). Both
horizons were comparable to 1988 pH determinations.

#1. Species and strain characterization. Species of soil
amoebae present at the study sites were isolated from soil

enrichment plates. So far no species differences have been noted between sites; species composition was the same as in previous years. Species included Acanthamoeba castellanii, A. polyphaga, A. astronyxis (small strain), Hartmannella sp., Rosculus sp., Naegleria gruberi, Vahlkampfia sp., and Mayorella sp. The isoenzyme analysis of genetic heterogeneity of A. polyphaga was not done in 1989.

#2. Population size and activity. As stated in previous annual reports, the number of replicate soil samples required to statistically compare soil amoeba populations between study sites was 8. From 1983 to 1985 soil amoeba populations increased from the start of the growing season to a peak in excess of a million amoebae/gram soil in August and then dropped sharply in September to a few thousand/gram soil. Vegetative amoebae formed a significant component of each monthly sample, including the smaller September and October populations. No differences were noted for a given soil horizon between the antenna, ground and control sites. The drought, beginning in 1986, has had a pronounced effect on population size, the ratio of vegetative to dormant cysts and some site differences in 1987 (the June and July counts. The results from the 1989 season obtained to date show population sizes characteristic of a drought year. Table 4 gives total counts of vegetative amoebae and cysts while Table 4A gives counts of cysts alone, thus the mathematical difference gives the number of vegetative amoebae present in a sample. Figure 1 interprets Tables 4 and 4A in showing total counts and the calculated percent vegetative amoebae by horizon and site at

various sampling dates. Table 4B demonstrates that the total population size in the organic horizon at the antenna site was greater than other sites for the July count; otherwise no significant differences were noted between sites for a given horizon and sampling date. As in past years cyst counts exhibited differences between sites which reflect the susceptibility of vegetative and cyst states to local conditions (e.g. food and moisture might explain these differences).

I have summarized the NOAA Climatological Data publications for monthly deviations from normal rainfall for 1985 through 1989 (Fig. 3) to emphasize the drought years. Soil moisture measurements indicate slightly drier soils during this period (Fig. 2), which may account for the effects of the drought on growth, although nutrient input from surface litter may be a more important component of limiting amoeba growth and would correlate with the rainfall pattern. In Fig. 3A the annual total rainfall is plotted together with the average maximum total amoeba population per year; this gives a good correlation between population size and annual rainfall.

#3. Growth and feeding activity. Growth experiments in soil submersible culture vessels were done over prolonged intervals in the 1989 field season. Past years have demonstrated that the technique is suitable, although prolonged incubation did reveal that corrosion between the electrode and the soldered wire attached to it was still a significant problem. The metal ions from corrosion leached into the saline, dramatically altering conductivity. This was eventually solved with better

polyurethane sealants. In 1989 we took glutaraldehyde-fixed

samples back to the lab for counting. Amoebae were counted

during active growth, which was a good deal cooler than the

temperatures normally used for laboratory isolates (e.g. 23 to

30° C). Cultures were left in the soil after growth reached its

maximum for another 2 weeks and then subcultured. In some cases

the cultures became contaminated with a small flagellate; then

subcultures were made from flagellate-free cultures. At the end

of the season, isoenzyme analyses were done on these amoebae. No

change in isoenzyme pattern was observed between the original

clone culture and subcultures grown in soil incubated at the

sites (Fig. 6). Growth rate data analysis is presented in Table

6 and indicated no difference between sites. For the 1989 growth

experiments I decided to use an excess of bacterial food to

support both maximum amoeba growth rate and maximum yield. Thus

vegetative amoebae persisted longer in the soil submersible

culture vessels than they would with limiting numbers of

bacteria. Analyses were done during exponential growth (Fig. 5)

to simplify statistical comparisons and to avoid growth limits

caused by a decrease in the bacterial food supply. If bacteria

decreased to the point where the growth of amoebae became

limiting, this would have been seen in Fig. 5 as a deceleration

in the growth rate. Comparing growth at 12' in the laboratory

(MGT = approx. 7.2 hr) with growth at 17' C (MGT = approx. 3.6

hr) (in 1988) brings up the need to track soil culture

temperature more closely. Temperatures have been the same

between study sites, but this is still an important variable if

this is not the case in future seasons. Again, comparisons of growth experiments done at different times of the year will certainly be affected by temperature which will differ over the season.

#4. Ambient monitoring. Table 5 (and Fig. 2) gives the mean % (w/w) moisture for individual measurements, taken when the soil was sampled. During the growing season (i.e. June, July and August) the soil was drier than in 1984 and 1985, roughly comparable to 1986 and 1987--both drought years as well.

Soil temperature recordings for the season (Fig.4) were comparable to previous field seasons (Fig.5).

7. Peer reviewers and publications:

I plan to use the following individuals as peer reviewers:

a. Prof. Thomas J. Byers

    Department of Molecular Genetics

    Ohio State University

b. Prof. Frederick L. Schuster

    Department of Biology

    Brooklyn College

Publications (1989):

1. Milligan, S.M. & Band, R.N. 1988. Rapid identification of species and strains of <u>Naegleria</u> with restriction digests of mitochondrial and plasmid DNA. Appl. & Environ. Microbiol. submitted for publication.

2. Attended V International Conference on Biology and Pathogenicity of Free Living Amoebae, Brussels, 1989. I chaired the session entitled Molecular Biology and Genetics of <u>Naegleria</u>. I Presented two papers: a. Identification of species and strains of <u>Naegleria</u> by analysis of restriction fragment length polymorphisms of plasmid and mitochondrial DNA, a simple diagnostic technique; b. Drought affects annual population size fluctuations and genetic diversity of soil amoeb.e. The proceedings of this meeting will be published in the J. Protozoology,.

4. In preparation: Seasonal fluctuations and drought effects on soil amoeba population size and genetic heterogeneity.

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TABLE 1. SOIL CHEMISTRY:[*]

<div align="center">SITE/HORIZON[**]</div>

ELEM.	DATE[***]	CO	AO	GO	CM	AM	GM
P	1	28,26	41,40	23,24	15,15	69,60	25,24
	2	23,23	37,41	21,22	18,14	77,67	23,25
K	1	148,132	136,124	148,124	36,36	45,40	36,36
	2	132,144	152,140	144,144	36,36	40,40	36,36
Ca	1	3160,2840	2640,2356	3040,2560	756,756	920,880	756,711
	2	2680,2960	2480,2600	2600,2760	960,920	756,920	800,756
Mg	1	214,214	140,160	253,236	80,80	88,89	80,84
	2	209,214	144,165	205,236	69,92	69,79	87,84
NO_3	1	4.9,4.8	4.0,4.0	4.3,3.9	3.8,4.0	3.3,3.6	4.1,4.9
	2	4.3,4.1	3.9,3.8	4.4,4.2	2.8,2.5	3.7,3.1	3.0,3.0
%Org.N.	1	6.4,6.3	7.0,5.1	5.5,5.2	1.5,1.2	1.5,1.7	1.8,1.7
	2	5.9,5.1	6.1,5.4	4.9,4.8	1.5,1.5	1.6,1.4	1.3,1.5

[*]Performed by Michigan State University Soil Testing Laboratory, data expressed as ppm except for %Org.N.

[**]SITE: C, control; A, antenna; G, ground.
   HORIZON: O, organic; M, mineral

[***]Data was obtained June 12 and Aug 12, 1989, each of which were taken from 20 random samples.

TABLE 2.   SOIL CHEMISTRY 2X ANOVA, between sites and dates:

ELEMENT			ORGANIC		MINERAL	
		D.F.	M.S.	F	M.S.	F
P	Site	2	347.58	181.35 **	3196.75	189.91 **
	Date	1	18.75	9.78 *	21.33	1.27 NS
	Interact.	2	1.75	0.91 NS	18.08	1.07 NS
	Error	6	1.92		16.83	
K	Site	2	4	0.04 NS	36.75	17.64 **
	Date	1	161.33	1.53 NS	2.08	1 NS
	Interact.	2	81.33	0.77 NS	2.08	1 NS
	Error	6	105.33		2.08	
Ca	Site	2	153748	3.47 NS	14517.75	5.12 NS
	Date	1	22188	0.5 NS	9240.75	3.26 NS
	Interact.	2	13188	0.29 NS	15219.75	5.36 *
	Error	6	44321.33		2838.08	
Mg	Site	2	6993.58	39.66 **	13.00	0.24 NS
	Date	1	161.33	0.92 NS	36.75	0.67 NS
	Interact	2	220.58	1.25 NS	93	1.7 NS
	Error	6	176.33		54.58	
NO_3	Site	2	0.36	16.65 **	0.24	2.32 NS
	Date	1	0.12	5.54 NS	2.61	25.7 **
	Interact.	2	0.18	8.42 *	0.6	5.91 *
	Error	6	0.02		0.1	
% Org.N	Site	2	0.88	2.18 NS	0.03	1.41 NS
	Date	1	0.91	2.25 NS	0.03	1.64 NS
	Interact.	2	0.08	0.19 NS	0.06	3.41 NS
	Error	6	0.4		0.02	

* = 5% significance level
** = 1% significance level

TABLE 3. SOIL pH:

DATE	SITE	HORIZON	MEAN pH ± S.E. (n = 10)
9JUN	Control	Organic	6.61 ± 0.06
		Mineral	6.41 ± 0.17
	Antenna	Organic	7.07 ± 0.13
		Mineral	6.79 ± 0.08
	Ground	Organic	7.08 ± 0.14
		Mineral	6.63 ± 0.11
2AUG	Control	Organic	6.23 ± 0.13
		Mineral	6.57 ± 0.09
	Antenna	Organic	6.7 ± 0.07
		Mineral	6.64 ± 0.08
	Ground	Organic	6.46 ± 0.15
		Mineral	6.57 ± 0.11

THREE WAY ANOVA, site, date, horizon:

		D.F.	M.S.	test#	F	
#1.	Site	2	1.141	1	8.431	**
#2.	Horizon	1	0.290	2	2.144	NS
#3.	Date	1	1.564	3	11.561	**
#4.	Site X Horizon	2	0.154	4	1.139	NS
#5.	Site X Date	2	0.170	5	1.257	NS
#6.	Horizon X Date	1	1.344	6	9.935	**
#7.	Site X Horizon X Date	2	0.081	7	0.596	NS
Error		108	0.135			

TWO WAY ANOVA, site, horizon for each date:

DATE		D.F.	M.S.	F	
6JUN	Site	2	1.004	6.65	**
	Horizon	1	1.442	9.56	**
	Interaction	2	0.082	0.54	NS
	Error	54	0.151		
2AUG	Site	2	0.307	2.55	NS
	Horizon	1	0.193	1.59	NS
	Interaction	2	0.153	1.27	NS
	Error	54	0.121		

** = 1% significance level

TABLE 4. Total counts from 8 samples per horizon/site:

SITE	HORIZON	DATE	MEAN #/g soil ± S.E.[*] (log#)	MEAN[**]
Control	Organic	6/19	3.2510 ± 0.0574	1,896
		7/17	3.5023 ± 0.1174	4,235
		8/14	4.1548 ± 0.1816	31,842
		9/12	3.8811 ± 0.2058	15,325
		10/15	3.3560 ± 0.1017	2,979
	Mineral	6/19	2.8674 ± 0.0489	769
		7/17	3.3009 ± 0.0641	2,162
		8/14	3.4678 ± 0.1524	4,447
		9/12	2.9249 ± 0.0682	917
		10/15	2.8636 ± 0.1205	1,075
Antenna	Organic	6/19	3.2294 ± 0.0777	1,879
		7/17	4.2059 ± 0.1597	21,346
		8/14	4.0351 ± 0.1878	21,947
		9/12	3.4824 ± 0.1430	4,548
		10/15	3.6665 ± 0.1338	6,666
	Mineral	6/19	2.7368 ± 0.0528	577
		7/17	3.3662 ± 0.1278	3,157
		8/14	3.2072 ± 0.0622	1,735
		9/12	2.9012 ± 0.0977	952
		10/15	2.8436 ± 0.1205	1,169
Ground	Organic	6/19	3.1575 ± 0.0683	1,574
		7/17	3.6423 ± 0.1130	5,432
		8/14	4.1852 ± 0.0981	18,234
		9/12	3.5597 ± 0.1322	5,013
		10/15	3.7225 ± 0.1462	7,624
	Mineral	6/19	2.7656 ± 0.0461	607
		7/17	3.2489 ± 0.0729	1,928
		8/14	3.3163 ± 0.0572	2,194
		9/12	2.9759 ± 0.1046	1,188
		10/15	2.8110 ± 0.0687	709

[*] Mean expressed as $\log_{10}$ number, used to calculate analysis of variance (Table 4B).

[**] Mean calculated from arithmetic data which will differ from converting the mean of log data to an arithmetic figure. The log of the arithmetic mean will not be the same as the mean of the log transformed data.

TABLE 4A. Cyst counts from 8 samples per horizon/site:

SITE	HORIZON	DATE	MEAN #/g soil ± S.E.* (log#)	MEAN**
Control	Organic	6/19	3.0135 ± 0.0465	1,085
		7/17	2.8622 ± 0.0263	738
		8/14	3.6858 ± 0.1001	5,759
		9/12	3.4446 ± 0.1993	5,395
		10/15	3.1480 ± 0.0897	1,612
	Mineral	6/19	2.6719 ± 0.0125	472
		7/17	2.7453 ± 0.0417	575
		8/14	3.0393 ± 0.0582	1,163
		9/12	2.9111 ± 0.0621	876
		10/15	2.8352 ± 0.1271	1,077
Antenna	Organic	6/19	3.0407 ± 0.0432	1,150
		7/17	3.2068 ± 0.0785	1,793
		8/14	4.2982 ± 0.1158	24,695
		9/12	3.1153 ± 0.0498	1,368
		10/15	3.2187 ± 0.1022	1,958
	Mineral	6/19	2.6570 ± 0.0164	457
		7/17	2.9240 ± 0.0764	936
		8/14	3.2311 ± 0.0373	1,751
		9/12	2.8494 ± 0.0683	778
		10/15	2.9428 ± 0.1345	1,374
Ground	Organic	6/19	3.0350 ± 0.0477	1,144
		7/17	3.5060 ± 0.0728	3,497
		8/14	3.7366 ± 0.1249	7,236
		9/12	3.2394 ± 0.1004	2,060
		10/15	3.1688 ± 0.0941	1,737
	Mineral	6/19	2.6474 ± 0.0311	456
		7/17	2.8160 ± 0.0449	679
		8/14	3.3865 ± 0.0678	2,626
		9/12	2.7738 ± 0.3810	611
		10/15	2.7399 ± 0.0978	710

* Mean expressed as $\log_{10}$ number, used to calculate analysis of variance (Table 4B).

** Mean calculated from arthmetic data which will differ from converting the mean of log data to an arithmetic figure.

TABLE 4B. One-way analysis of variance by date and horizon. Data log transformed (see Table 4 & 4A).

TOTAL COUNT

HORIZON	DATE	GROUPS	DF	MS	F
ORGANIC	6/19	among	2	0.0191	0.5129 NS
		within	21	0.0373	
	7/17	among	2	1.1098	7.9962 **
		within	21	0.1388	
	8/14	among	2	0.0504	0.2426 NS
		within	21	0.2077	
	9/12	among	2	0.3577	1.6702 NS
		within	21	0.2141	
	10/15	among	2	0.3118	0.1193 NS
		within	21	0.1323	
MINERAL	6/19	among	2	0.0377	1.9351 NS
		within	21	0.0195	
	7/17	among	2	0.0277	0.4027 NS
		within	21	0.0687	
	8/14	among	2	0.1371	1.6928 NS
		within	21	0.0810	
	9/12	among	2	0.0117	0.1743 NS
		within	21	0.0670	
	10/15	among	2	0.0056	0.9491 NS
		within	21	0.1077	

CYST COUNT

HORIZON	DATE	GROUPS	DF	MS	F
ORGANIC	6/19	among	2	0.0019	0.0979 NS
		within	21	0.0189	
	7/17	among	2	0.8303	25.6201 **
		within	21	0.0324	
	8/14	among	2	0.9241	8.8761 **
		within	21	0.1041	
	9/12	among	2	0.2212	1.5864 NS
		within	21	0.1394	
	10/15	among	2	0.0105	0.1446 NS
		within	21	0.0729	
MINERAL	6/19	among	2	0.0014	0.3263 NS
		within	21	0.0042	
	7/17	among	2	0.0648	2.5338 NS
		within	21	0.0256	
	8/14	among	2	0.2421	9.6847 **
		within	21	0.0250	
	9/12	among	2	0.0379	1.4233 NS
		within	21	0.0266	
	10/15	among	2	0.0824	0.7056 NS
		within	21	0.1168	

* = 5% significance level
** = 1% significance level

TABLE 5. SOIL MOISTURE (%, w/w)[1]

	CONTROL SITE		ANTENNA SITE		GROUND SITE	
HORIZON	ORG	MIN	ORG	MIN	ORG	MIN
DATE						
6/19	34.4 ± 6	16.9 ± 1.7	32.0 ± 4.5	14.9 ± 1.7	33.9 ± 4.0	16.0 ± 2.4
7/17	24.5 ± 6.8	11.4 ± 2.1	21.0 ± 5	9.0 ± 1	24.0 ± 5	11.0 ± 1.6
8/14	32 ± 4.7	17.2 ± 1.8	31.2 ± 4.3	12.7 ± 2.2	28.4 ± 4	14.8 ± 1.8
9/12	43.3 ± 8.3	15.2 ± 1.5	48.5 ± 5.6	11.9 ± 1.7	40.8 ± 6.9	12.0 ± 1/3
10/15	28 ± 7.8	12.6 ± 1.8	34.0 ± 5	6.5 ± 1.4	35.0 ± 7.7	9.4 ± 1.7

ONE WAY ANOVA (between sites):

		ORGANIC		MINERAL	
Date		D.F	M.S.	D.F.	M.S.
6/19	Between	2	12.83	2	8.03
	Within	21	24.08	21	3.85
	F=		0.53 NS		2.09 NS
7/17	Between	2	28.67	2	13.23
	Within	21	32.08	21	2.66
	F=		0.89 NS		4.98 *
8/14	Between	2	28.59	2	40.56
	Within	21	18.06	21	3.77
	F=		1.52 NS		10.75 **
9/12	Between	2	123.44	2	28.65
	Within	21	49.29	21	2.28
	F=		2.5 NS		12.58 **
10/15	Between	2	114.67	2	74.48
	Within	21	48.38	21	2.69
	F=		2.37 NS		27.62 **

1 = mean ± S.D. (n=8)
* = 5% significance level
**= 1% significance level

TABLE 6. Regression calculations tor growth of <u>Acanthamoeba</u> <u>polyphaga</u> in soil submersible culture vessels, data log transformed.

Details of electric currents in vessels given in Table 7. Actual operating schedules of the ELF antenna will be collated from IITRI data.

*Date	Experiment*	Slope[++]	95% Confidence Limits[+++]
6/14/89	E-Field, control	0.02333	L1 = 0.01004 / L2 = 0.03666
	" , antenna	0.02465	L1 = 0.00252 / L2 = 0.04679
	" , ground	0.02272	L1 =-0.00278 / L2 = 0.04819
	Current, control	0.02008	L1 = 0.01714 / L2 = 0.05733
	" , antenna	0.01716	L1 = 0.00165 / L2 = 0.03602
	" , ground	0.02113	L1 =-0.00088 / L2 = 0.04308
7/11/89	E-Field, control	0.01106	L1 = 0.00502 / L2 = 0.01702
	" , antenna	0.00869	L1 = 0.00459 / L2 = 0.01285
	" , ground	0.01209	L1 = 0.00738 / L2 = 0.01681
	Current, control	0.00775	L1 = 0.00296 / L2 = 0.01254
	" , antenna	0.00721	L1 = 0.00083 / L2 = 0.01359
	" , ground	0.00812	L1 = 0.00532 / L2 = 0.01092
8/18/89	E-Field, control	0.02922	L1 = 0.00781 / L2 = 0.05063
	" , antenna	0.02886	L1 = 0.00992 / L2 = 0.04779
	" , ground	0.02901	L1 = 0.00529 / L2 = 0.05273
	Current, control	0.02723	L1 = 0.00488 / L2 = 0.04959
	" , antenna	0.02912	L1 = 0.01252 / L2 = 0.04573
	" , ground	0.02588	L1 = 0.00633 / L2 = 0.04544

* Three replicate experiments were done both E-field and Current density experiments at each site. Duplicate counts were done on each culture.

[++] Mean generation times were: 16 to 18 hr.

[+++]For the slope of the curve; Bonferoni T-tests of slopes:

		E. Field	Current Density
6/14/89	Control vs. Antenna	0.22049	0.30074
	Control vs. Ground	0.09155	0.10468
	Antenna vs. Ground	0.24602	0.59051
7/11/89	Control vs. Antenna	1.01051	0.21571
	Control vs. Ground	0.49665	0.12962
	Antenna vs. Ground	1.72073	0.41639
8/18/89	Control vs. Antenna	0.03952	0.21597
	Control vs. Ground	0.02042	0.13367
	Antenna vs. Ground	0.01573	0.40091

14 d.f. for error; no significant differences were noted.

TABLE 7a. Culture cell currrent densities and E-field voltages measured during growth experiments (Table 6) for June 14, 1989.

Electrodes[1]	Voc (mv)	Vcl (mv)[4]	Vr (mv)	Ecl (mv/m)[2]	Jcl (ma/m²)[3]
Control,CD:					
1	0.98	*	0.96	*	0.002
2	0.92	*	0.95	*	0.002
3	1.09	*	1.07	*	0.003
Control,EF:					
1	0.49	0.12	*	1.06	*
2	0.60	0.12	*	1.06	*
3	0.76	0.12	*	1.06	*
Antenna,CD:					
1	51	*	51	*	0.13
2	43	*	43	*	0.14
3	56	*	56	*	0.14
Antenna,EF:					
1	15	6.4	*	56.6	*
2	17	6.4	*	56.6	*
3	20	6.4	*	56.6	*
Ground,CD:					
1	14	*	14	*	0.036
2	17	*	17	*	0.043
3	17	*	17	*	0.043
Ground,EF:					
1	7	2.00	*	17.7	*
2	10	2.00	*	17.7	*
3	10	2.00	*	17.7	*

[1]CD = current density cultures; EF = E-field cultures.

[2]E-field: Ecl (mv/m) = Vcl / 0.113 (length between electrodes).

[3]Current density: Jcl (mA/m²) = Vr / R * xs. area of cl (m²), where R (ohms) = 2.5 * 10⁶ for J; 100 for E. Area of cl varied depending on submerged electrode area; for this experiment it was 1.57 * 10⁻⁴ m².

[4]Vcl for EF adjusted to this value, calculated: E (1m) * 0.113 (length between electrodes).

*Value too low for meter to accurately record.

TABLE 7b. Culture cell currrent densities and E-field voltages measured during growth experiments (Table 6) for July 11, 1989.

Electrodes[1]	Voc (mv)	Vcl (mv)[4]	Vr (mv)	Ecl (mv/m)[2]	Jcl (ma/m²)[3]
Control,CD:					
1	0.53	*	1.07	*	0.0027
2	0.58	*	0.94	*	0.0024
3	0.58	*	1.04	*	0.0026
Control,EF:					
1	1.00	0.14	*	1.2	*
2	0.86	0.14	*	1.2	*
3	1.12	0.14	*	1.2	*
Antenna,CD:					
1	32	*	32	*	0.082
2	27	*	27	*	0.069
3	33	*	33	*	0.084
Antenna,EF:					
1	16	3.76	*	33.2	*
2	16	3.76	*	33.2	*
3	16	3.76	*	33.2	*
Ground,CD:					
1	17	*	17	*	0.043
2	17	*	17	*	0.043
3	18	*	18	*	0.046
Ground,EF:					
1	20	2.13	*	18.8	*
2	12	2.13	*	18.8	*
3	11	2.13	*	18.8	*

[1]CD = current density cultures; EF = E-field cultures.

[2]E-field: Ecl (mv/m) = Vcl / 0.113 (length between electrodes).

[3]Current density: Jcl (mA/m²) = Vr / R * xs. area of cl (m²), where R (ohms) = 2.5 * 10⁵ for J; 100 for E. Area of cl varied depending on submerged electrode area; for this experiment it was $1.57 * 10^{-4}$ m².

[4]Vcl for EF adjusted to this value, calculated: E (1m) * 0.113 (length between electrodes).

*Value too low for meter to accurately record.

TABLE 7c. Culture cell currrent densities and E-field voltages measured during growth experiments (Table 6) for August 18, 1989.

Electrodes[1]	Voc (mv)	Vcl (mv)[4]	Vr (mv)	Ecl (mv/m)[2]	Jcl (ma/m^2)[3]
Control,CD:					
1	1.43	*	1.44	*	0.0037
2	1.3	*	1.29	*	0.0033
3	1.57	*	1.64	*	0.0042
Control,EF:					
1	1.4	0.2	*	1.77	*
2	1.58	0.2	*	1.77	*
3	1.5	0.2	*	1.77	*
Antenna,CD:					
1	53	*	53	*	0.13
2	44	*	44	*	0.11
3	54	*	54	*	0.14
Antenna,EF:					
1	^;	6.18	*	54.7	*
2	94	6.18	*	54.7	*
3	71	6.18	*	54.7	*
Ground,CD:					
1	22	*	22	*	0.056
2	21	*	21	*	0.054
3	24	*	24	*	0.061
Ground,EF:					
1	27	2.72	*	24.1	*
2	36	2.72	*	24.1	*
3	14	2.72	*	24.1	*

[1]CD = current density cultures; EF = E-field cultures.

[2]E-field: Ecl (mv/m) = Vcl / 0.113 (length between electrodes).

[3]Current density: Jcl (mA/m^2) = Vr / R * xs. area of cl (m^2), where R (ohms) = 2.5 * 10^6 for J; 100 for E. Area of cl varied depending on submerged electrode area; for this experiment it was 1.57 * 10^{-4} m^2.

[4]Vcl for EF adjusted to this value, calculated: E (1m) * 0.113 (length between electrodes).

*Value too low ror meter to accurately record.

Figure 1A. Summary of soil counts for 1989.
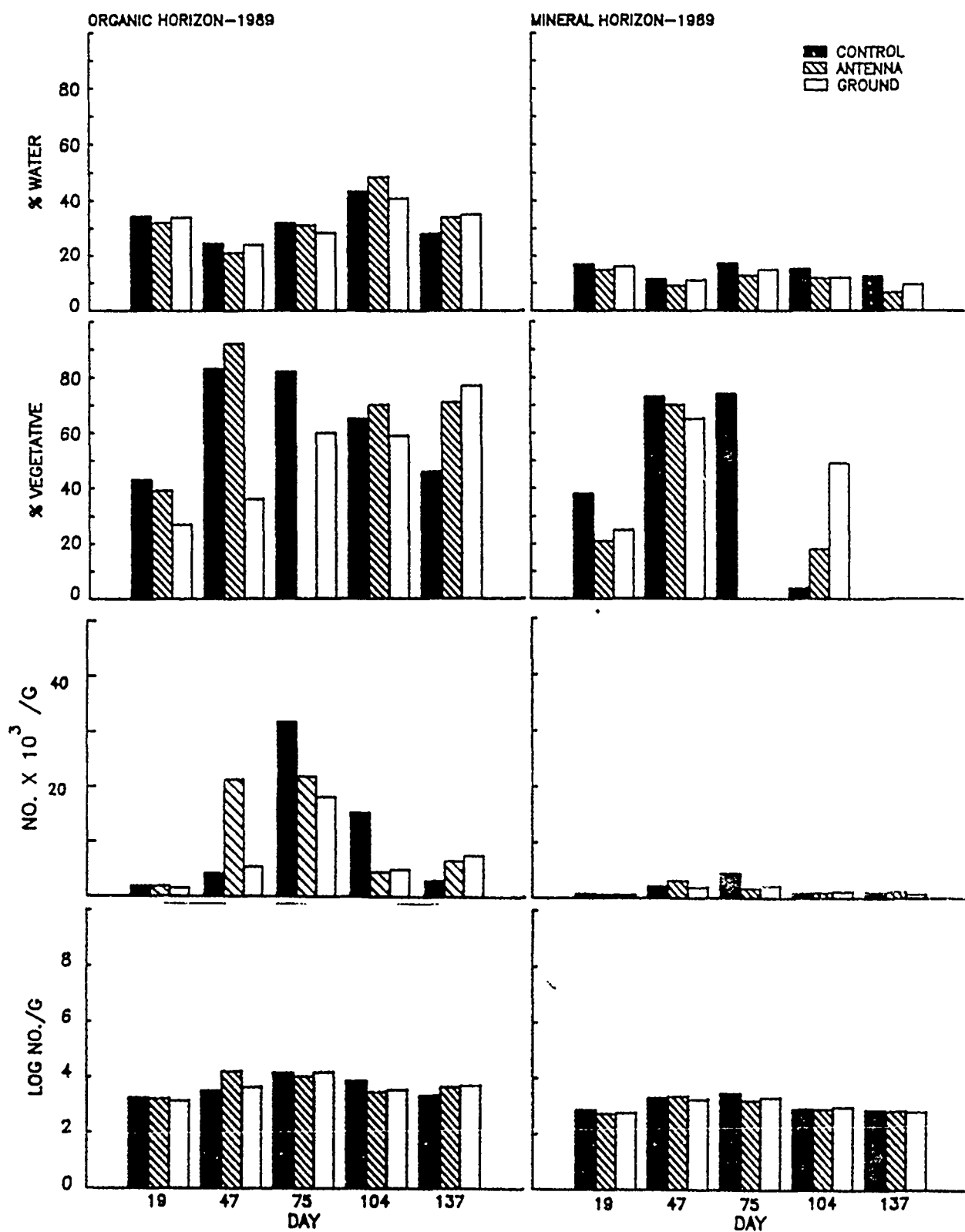
Figure 1B. Summary of previous years.

ORGANIC HORIZON - 1984

MINERAL HORIZON - 1984
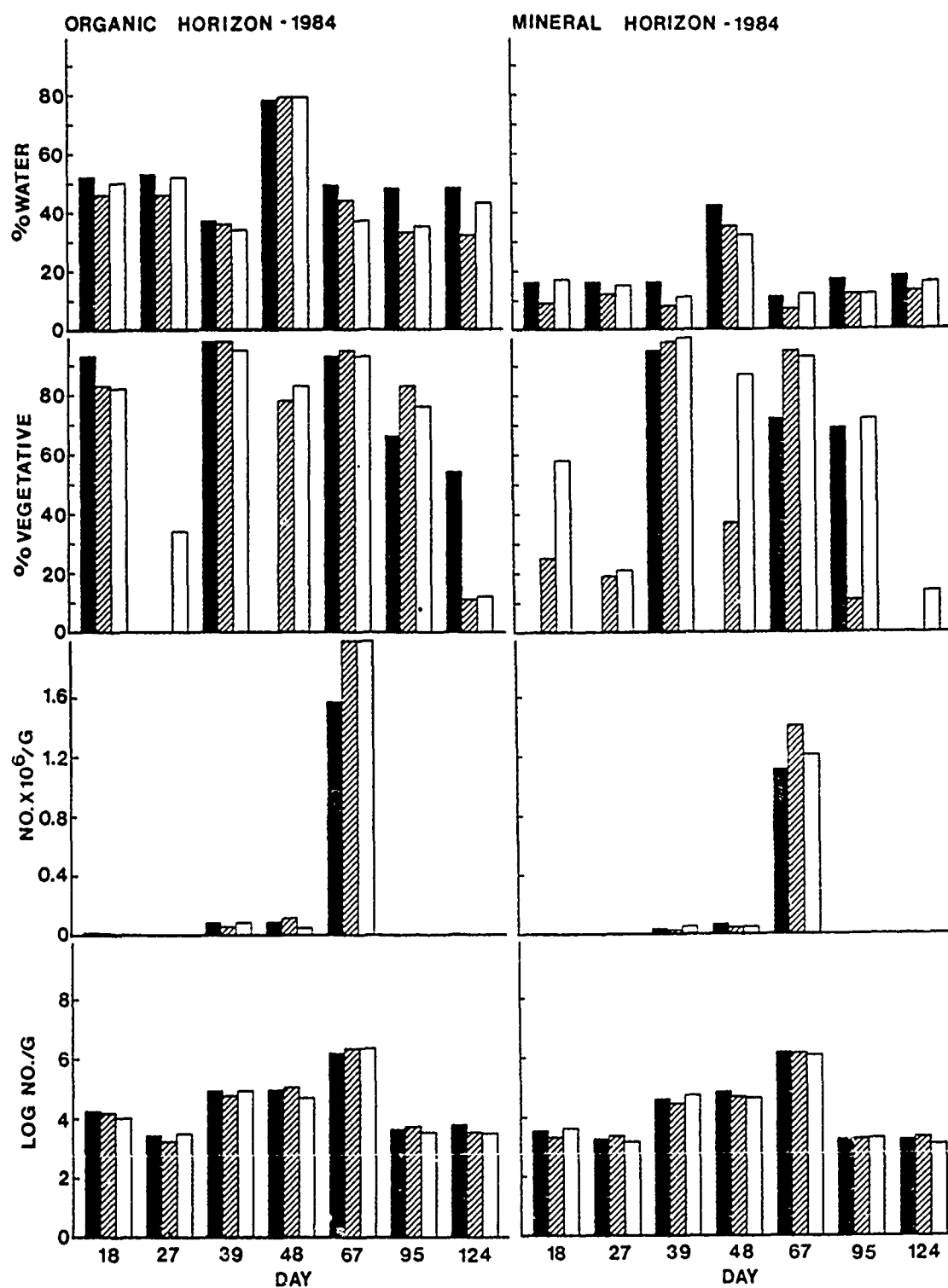
Figure 1C.   Summary of previous years.

ORGANIC HORIZON-1985   MINERAL HORIZON-1985
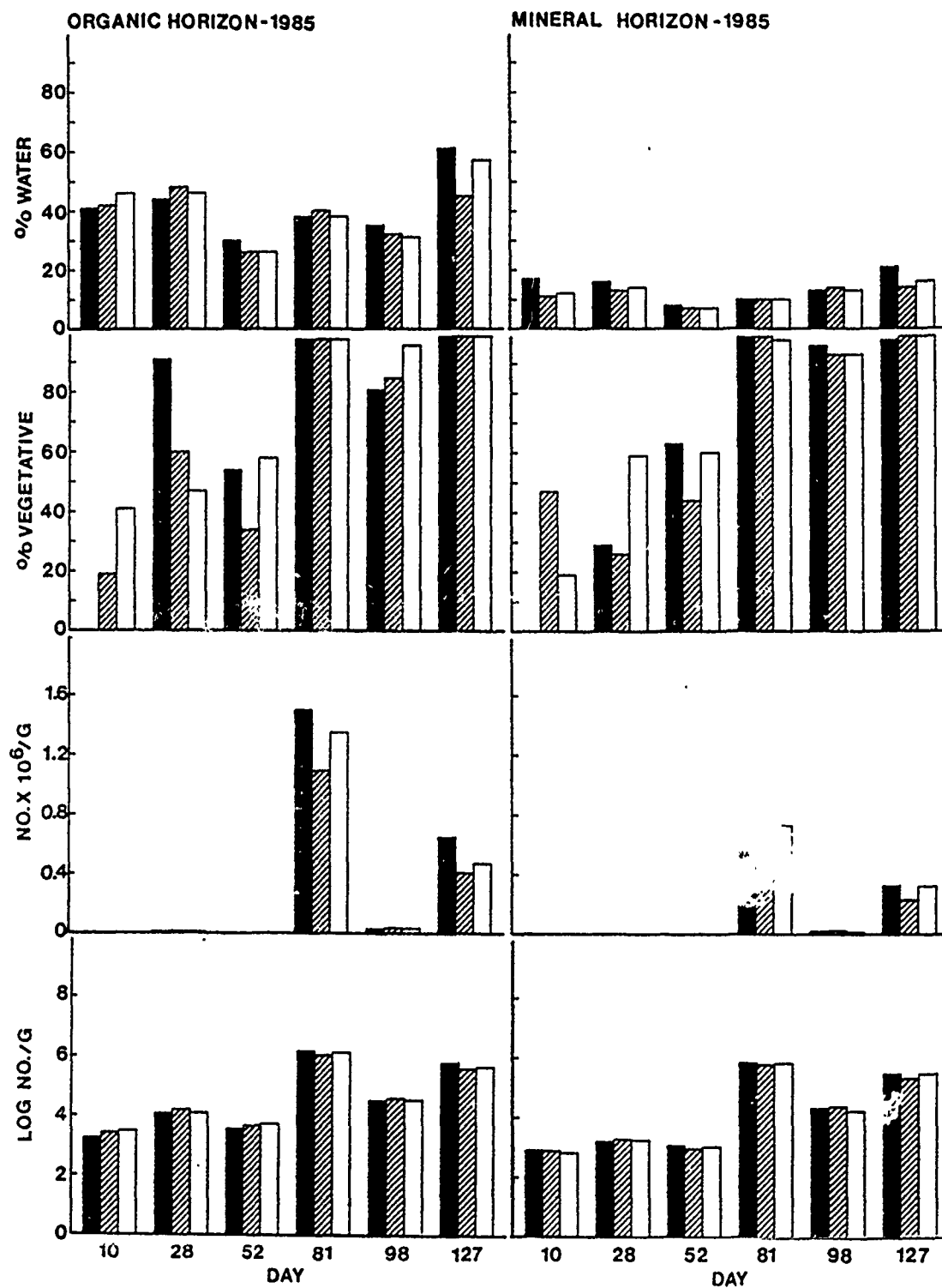
Figure 1D. Summary of previous years.

ORGANIC HORIZON-1986   MINERAL HORIZON-1986
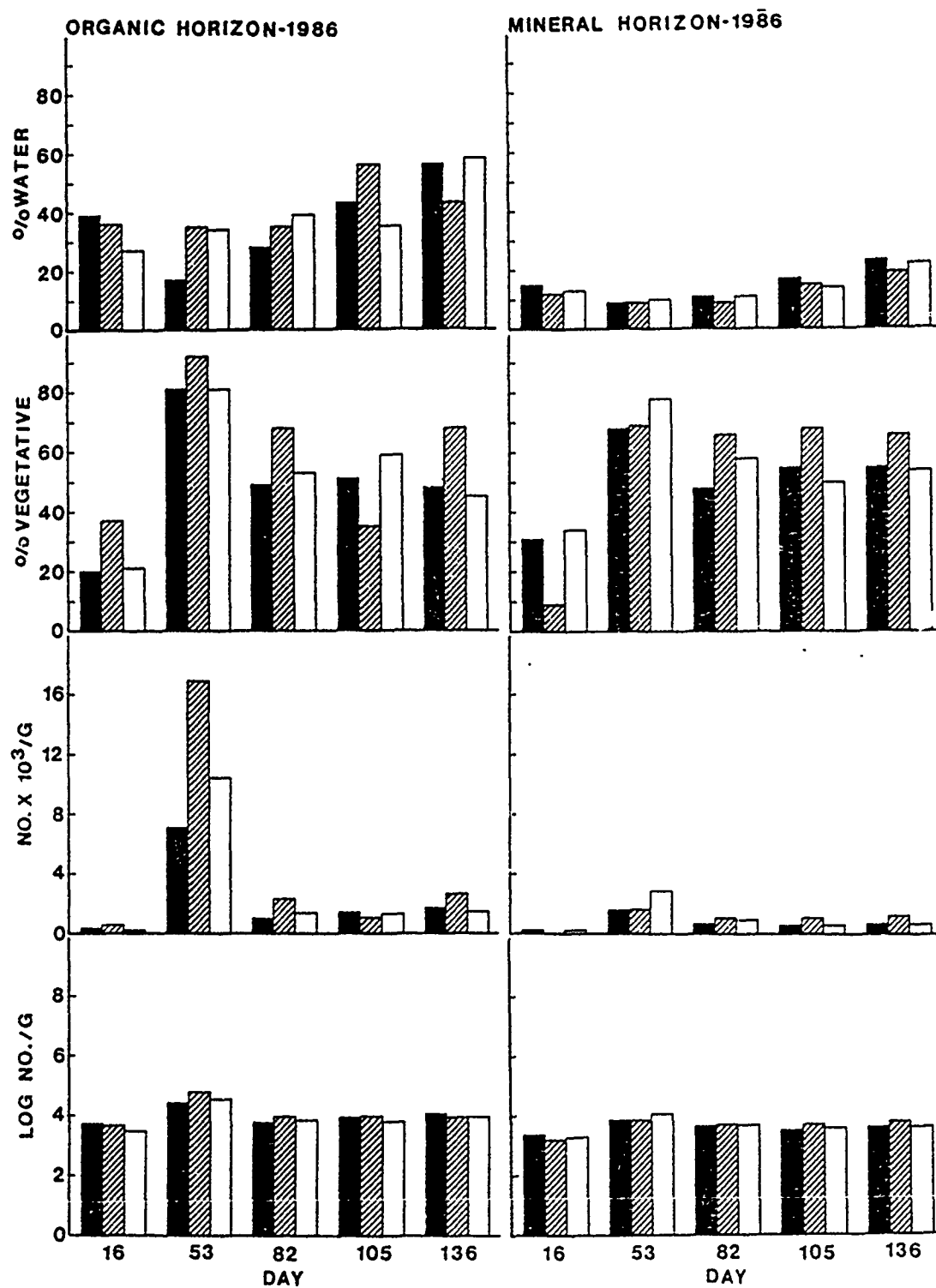
Figure 1E.   Summary of previous years.
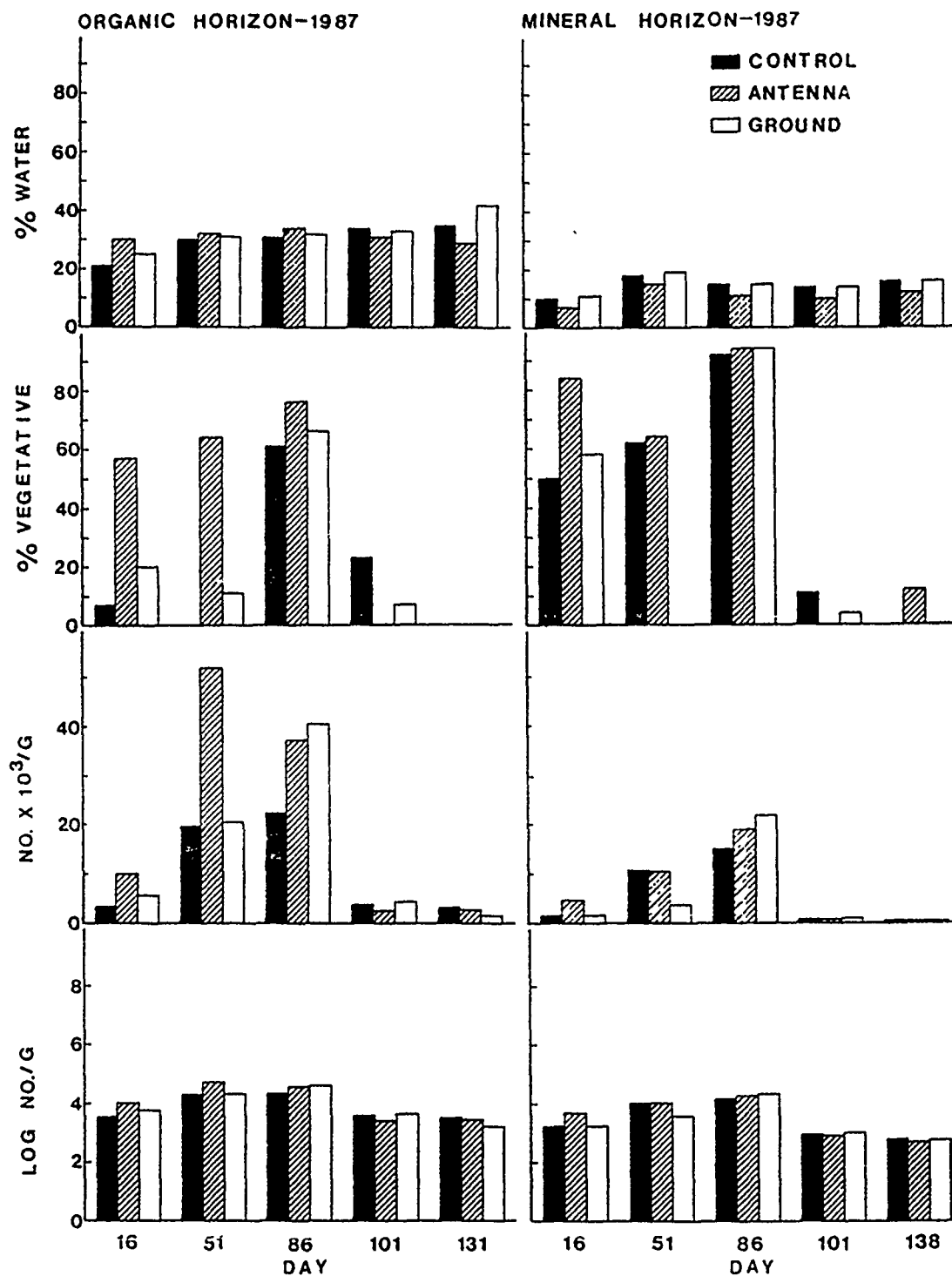
Figure 1F. Summary of previous years.
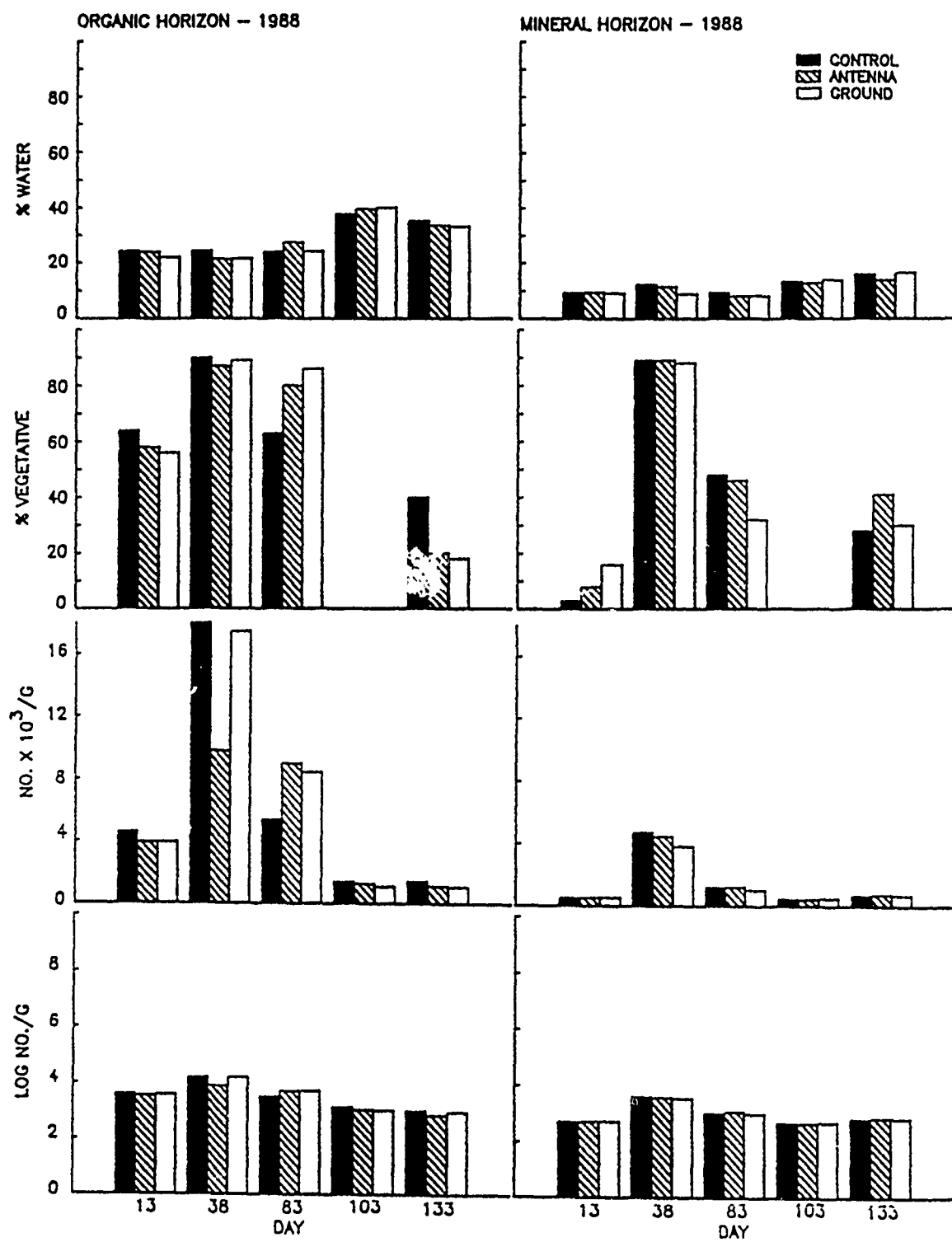
Figure 2. Moisture content of soil samples taken for counting amoebae.

MEAN SOIL MOISTURE
ORGANIC HORIZON

MINERAL HORIZON

JUN   JUL   AUG   SEP   OCT

1984   1985   1986   1987   1988
1989
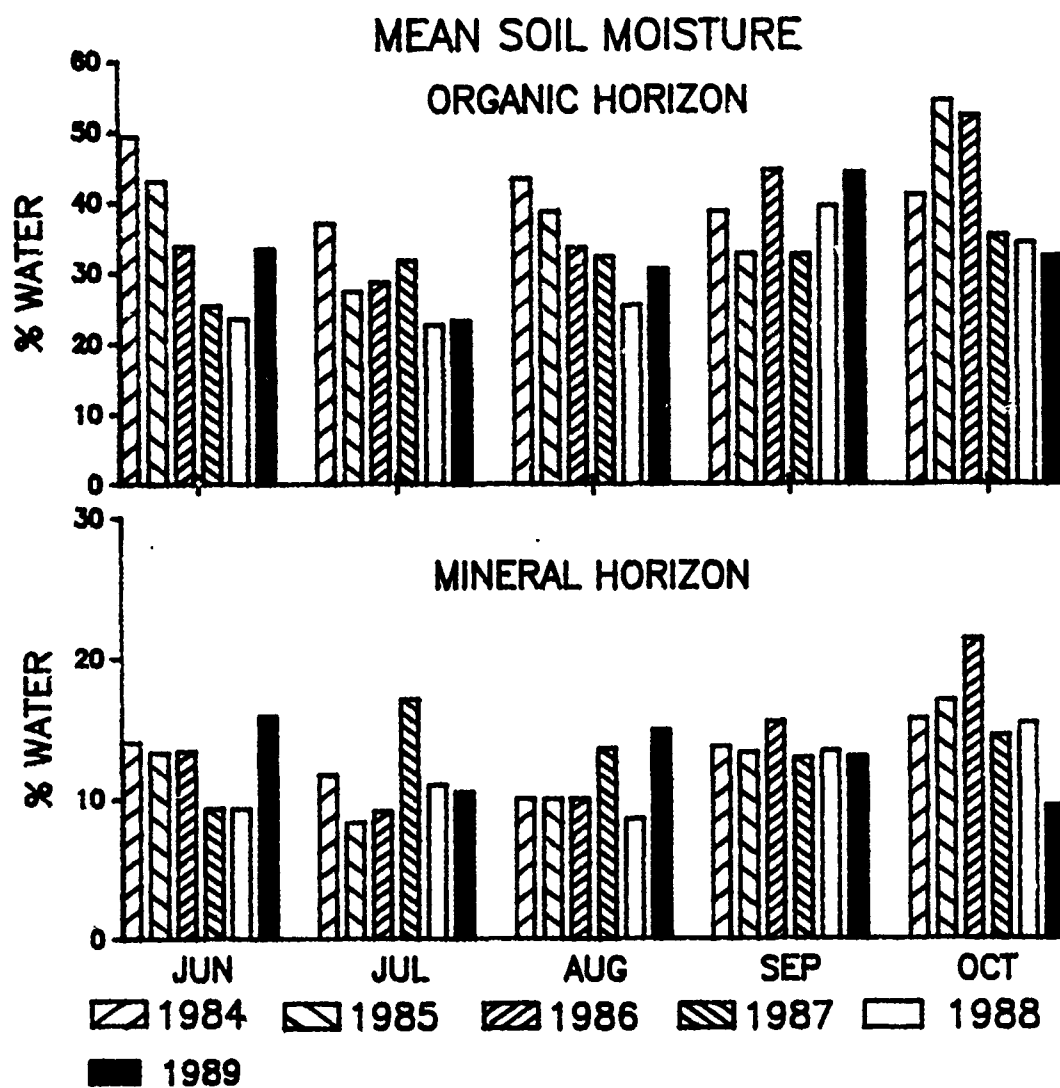
Figure 3. Annual rainfall departure from normal for 1984 & 1985 (normal rain) and for 4 drought years (1986 through 1989).

DEPARTURE FROM NORMAL RAINFALL
NOAA DATA, IRON MOUNTAIN

PLOTTED BY MONTH FOR EACH YEAR

Figure 3A.

ANNUAL DEPARTURE FROM NORMAL

Data from NOAA CD for Iron Mountain, Michigan

AVG MAX TOTAL AMOEBAE / G SOIL
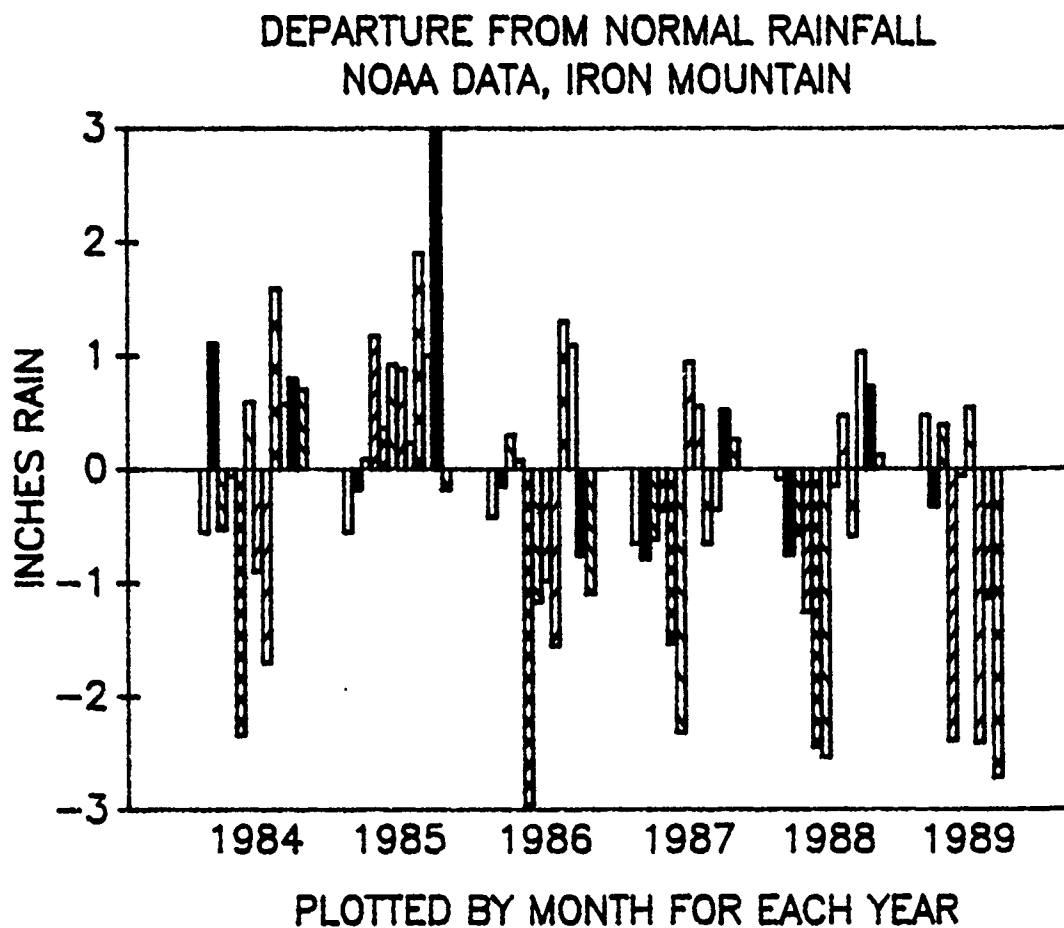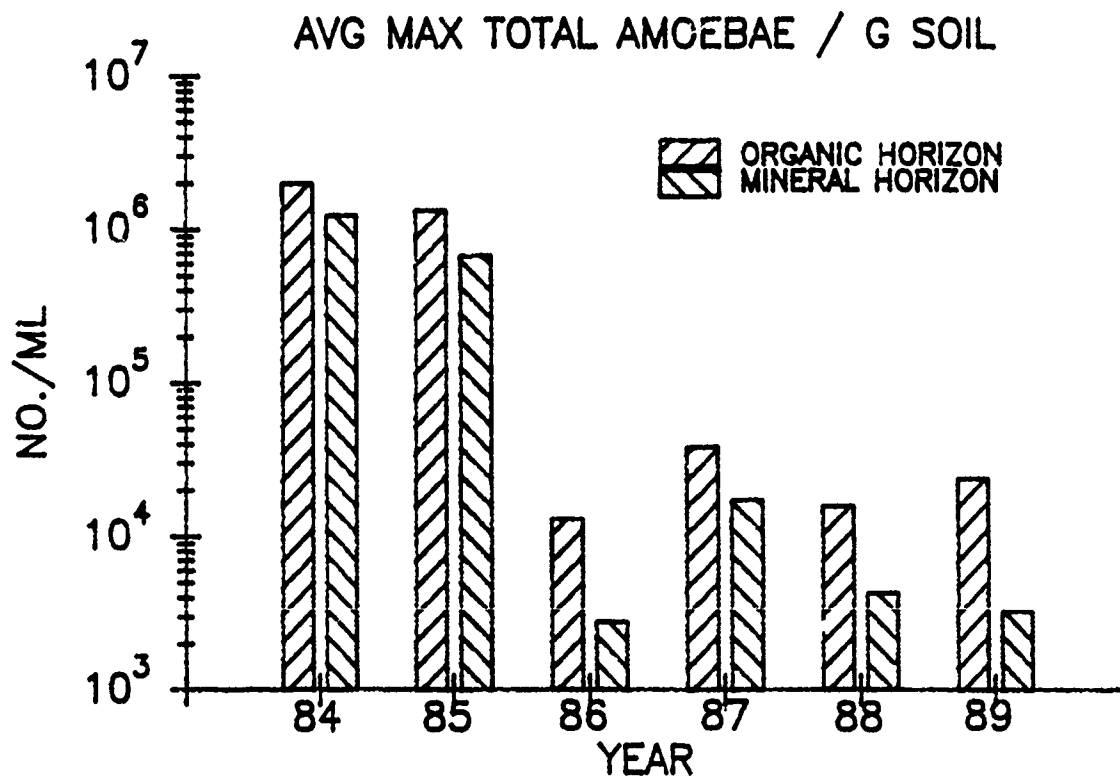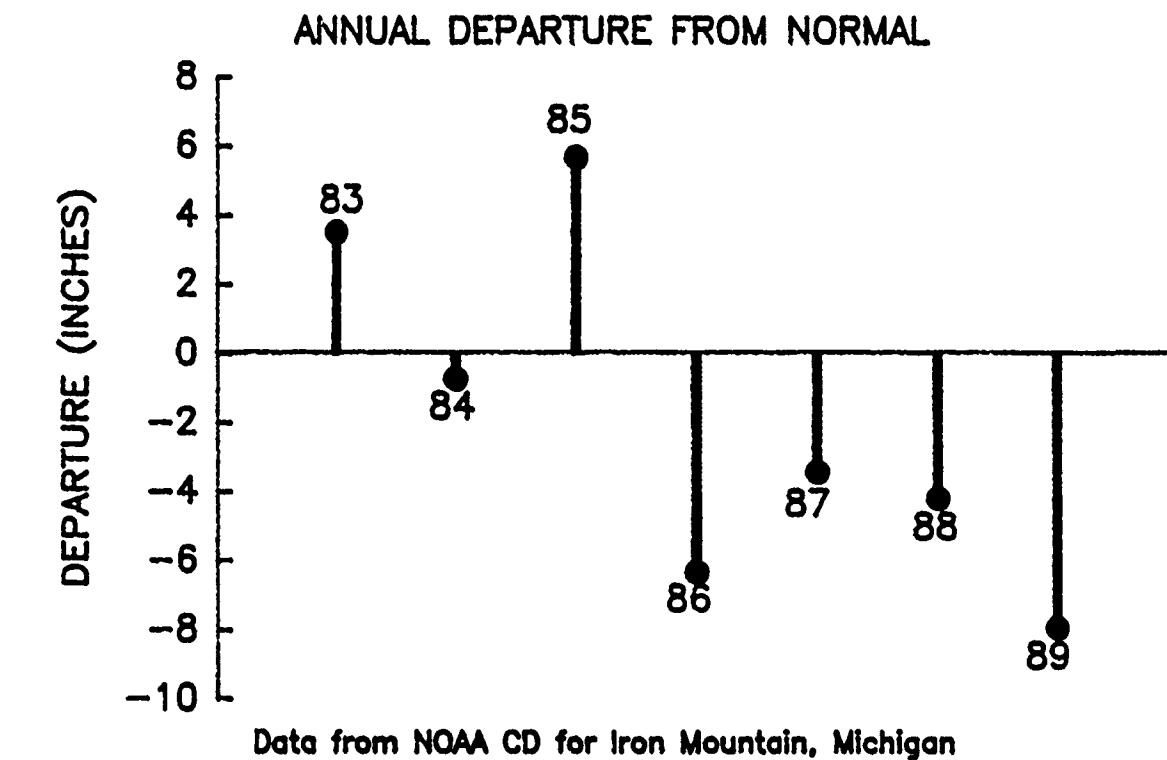
Figure 4. Pooled temperature records showing mean daily temperatures with S.D. error bars, plotted every third day.

MEAN SOIL TEMPERATURE
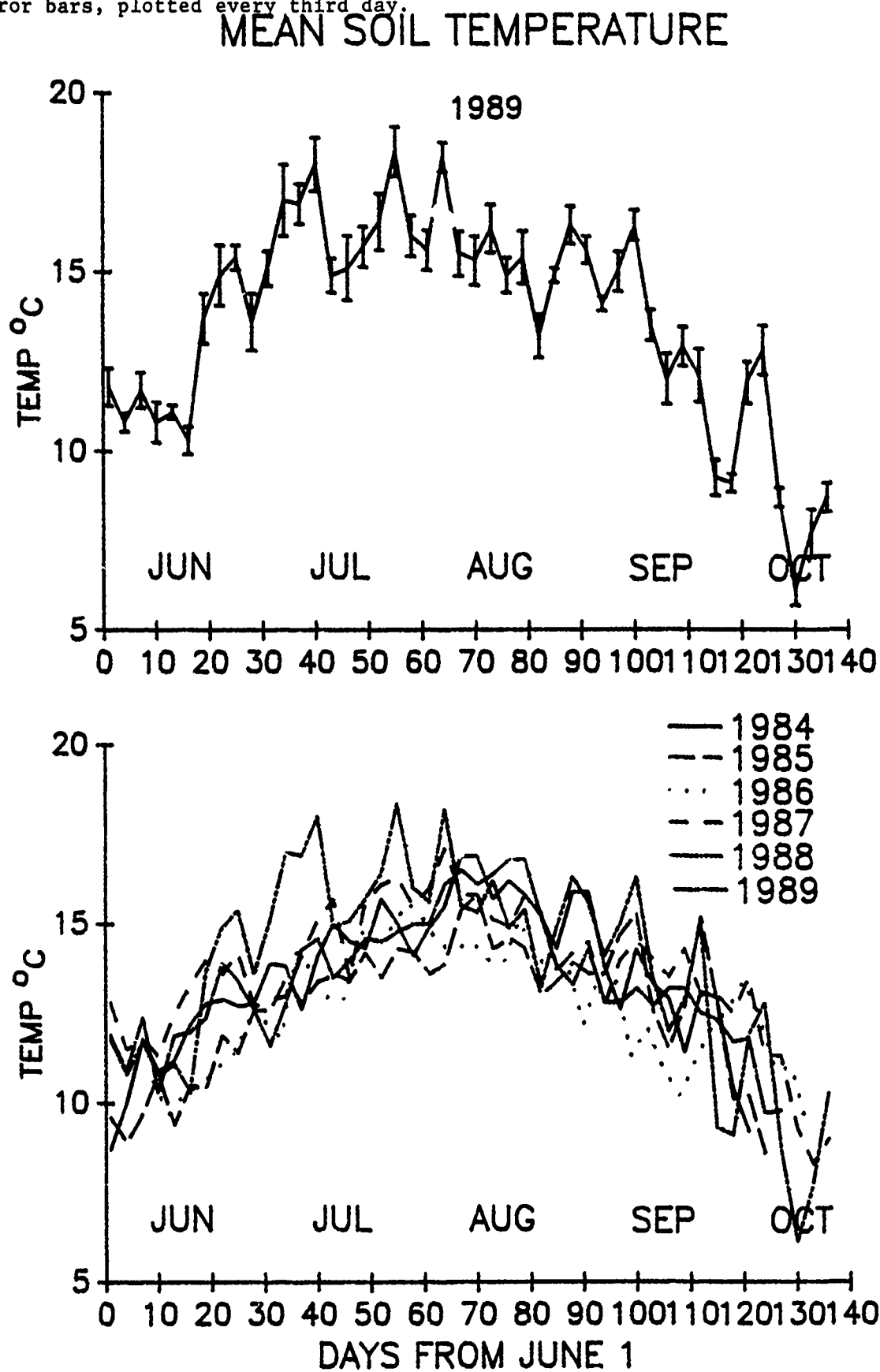
Figure 5. Growth of _Acanthamoeba polyphaga_ in soil submerged cultures.

T-TUBE GROWTH

Legend:
- • = ANT, CURR
- ♦ = ANT, E-FLD
- ▲ = GND, CURR
- ▲ = GND, E-FLD
- ■ = CON, CURR
- ▼ = CON, E-FLD

JUNE 14, 1989

JULY 11, 1989

AUGUST 18, 1989

HOURS
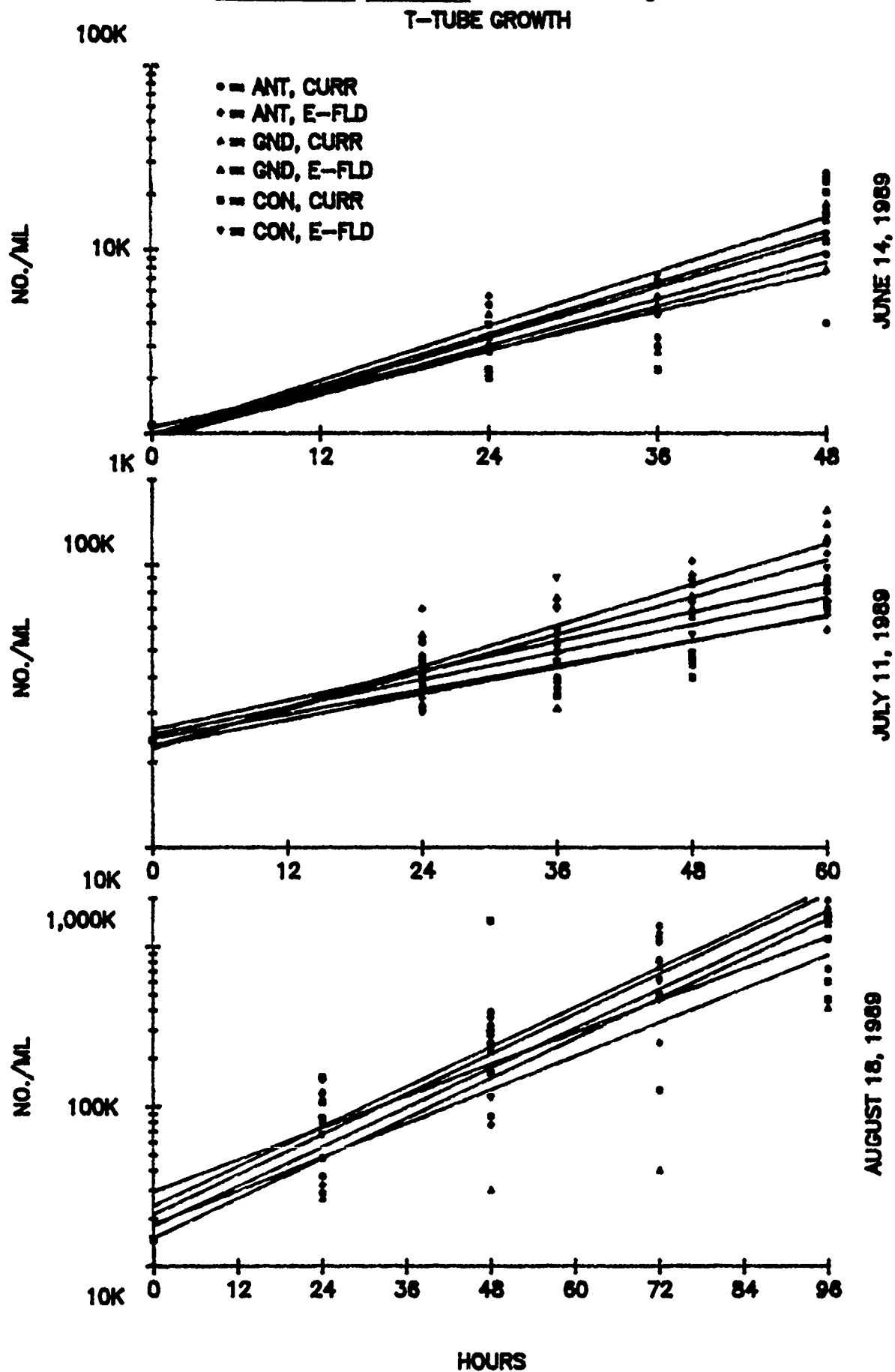
Figure 6. Isozyme patterns for <u>Acanthamoeba polyphaga</u> clone used in soil submerged cultures.

Isozyme patterns

1 = ME
2 = PGM
3 = ICD
4 = HK

5 = BE-2
6 = 6-PGD
7 = PE-1
8 = AE
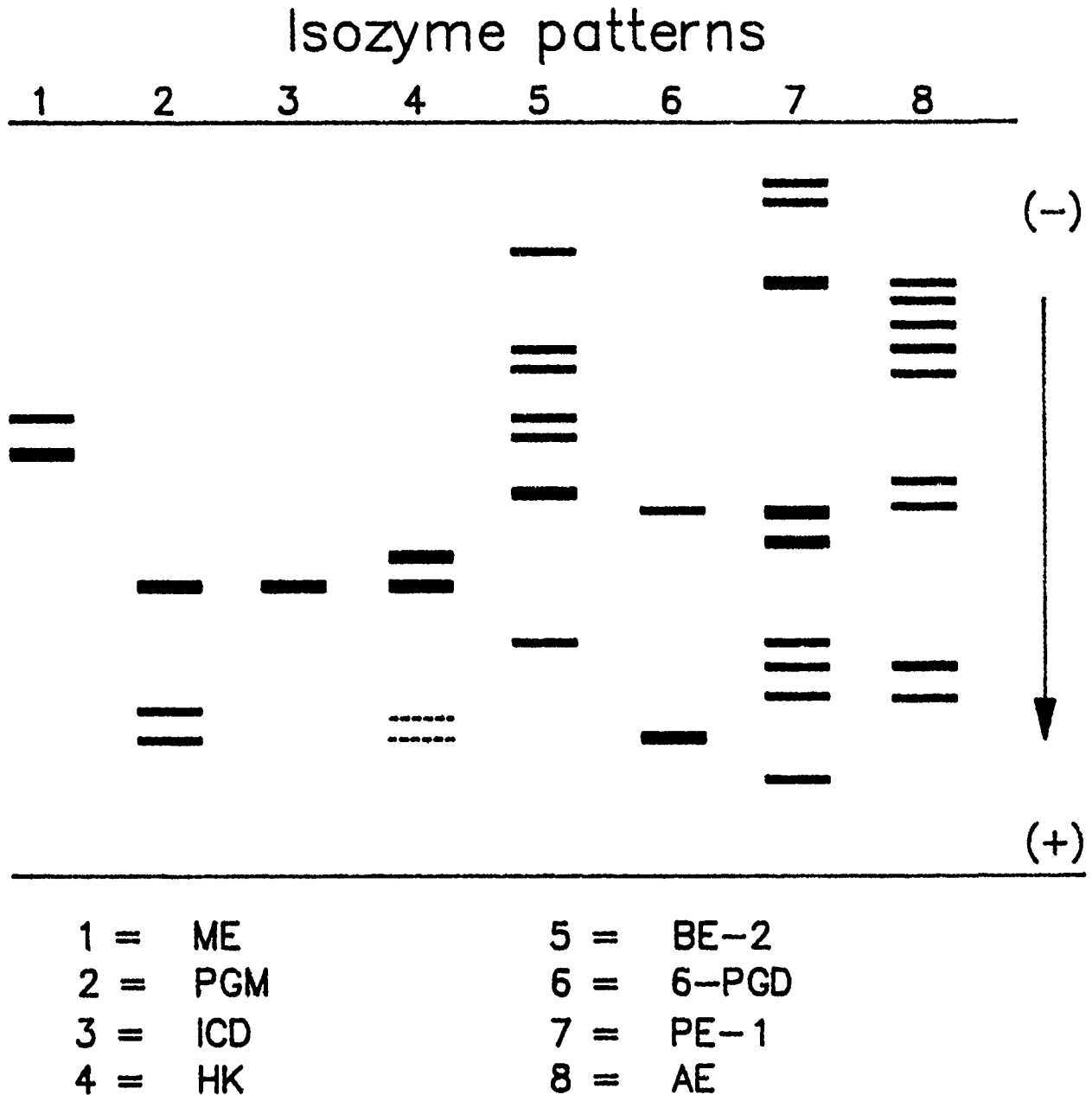
ME = malic enzyme
PGM = phosphoglucomutase
ICD = isocitrate dehydrogenase
HK = hexokinase
BE-2 = butyryl esterase-2
6-PGD = 6-phosphogluconate dehydrogenase
PE-1 =propionyl esterase
AE = acetyl esterase

Subcontractor: Michigan State University

East Lansing, Michigan 48824

Subcontract No. E06595-88-C-004
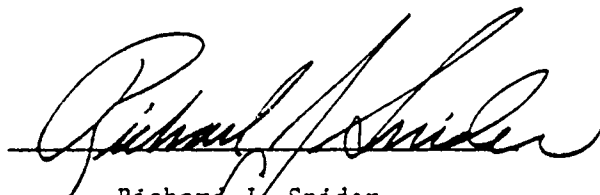
ELF Communications System Ecological Monitoring Program:
Arthropoda and Earthworms
Tasks 5.3. and 5.4.

Annual Report

1989

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Subcontract No. E06595-88-C-004

ELF Communications System Ecological Monitoring Program:

Arthropoda and Earthworms

Tasks 5.3. and 5.4.

<u>ANNUAL REPORT</u>

<u>1989</u>

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ABSTRACT

Sampling protocols and schedules of previous years were adhered to
in 1989: diel pit-trapping at weekly intervals, arthropod and earthworm
sampling at intervals of two weeks from early May to late October.

Diversity of collembolan communities proved to be consistently higher
in Test, and decreased gradually in both sites from year to year. Seasonal
abundance fluctuations of most arthropod species remain too variable for
valid between-site testing, but mean annual abundances of several species
of mites and Collembola are well correlated between Test and Control.
Patterns of development of indicator arthropods, measured by seasonal
frequencies of developmental stages, have been well synchronized between
sites. Most surface-active arthropods, in which numbers captured depend
on physiological state (mainly reproductive activity in beetles and mites)
as well as on environmental conditions, have shown well-correlated activity
patterns in both sites.

Vertical distribution of litter- and soil-dwelling earthworms was again
dependent mainly on substrate moisture, with the exception of one species
in Test: in that species, ambiguous results may have been caused by unusual
population structure due to low reproduction in 1988. Seasonal patterns
of reproduction and growth for all four species of interest are now well
documented. Litter-dwellers take one to two years, soil dwellers at least
two years to reach maturity. The smallest litter-inhabiting species tends
to die after reproducing, all others appear to reproduce more than once over
a period of two to several years after reaching adulthood. Several life
history parameters dealing with reproduction as well as behavioral responses
to environmental conditions have shown good correlation between Test and

Control.

Litter inputs in both sites did not differ from previous years. Seasonal leaf litter standing crops, typically slightly higher in Control during mid-summer, did not differ significantly between sites. Turnover rate estimates for natural forest floor litter were unusually high in Control in 1989; we suspect that unusually low numbers of a major earthworm decomposer species were the cause. Controlled decomposition experiments (litterbags) show that rates of litter breakdown in Test were consistent with previous years. Expanded decomposition studies were implemented in November of 1989 and will be repeated throughout the remaining project years.

SUMMARY

Sampling and extraction protocols were continued as in previous years.
Earthworm and arthropod populations of litter and soil were sampled at
intervals of two weeks from May 8 to October 23, 1989. Diel activity of
arthropods was monitored at weekly intervals by means of pit-traps. Air
and soil temperature, rainfall, and soil and litter moisture were also
monitored throughout the season.

Arthropod data are available through 1988, 1989 species identification
being as yet incomplete. All data pertaining to earthworms are at hand.
Two data bases, added to project goals relatively recently, are still
incomplete but will be available by mid-1990: population structure of
Isotoma notabilis, which requires size measurements of all individuals
extracted from litter and soil; and fecundity estimates for carabid beetles,
currently restricted to two species and three years.

In keeping with previous reports, we summarize results according to
three categories:

1. Abundance of animals, and its seasonal and yearly fluctuations.

For arthropod populations, mean annual abundances of several taxa have
proven most useful, continuing to fluctuate in parallel in Test and Control
(I. notabilis, S. henshawi and two species of mites). In the two collembolans,
seasonal increases and decreases were also significantly correlated.

Diversity indices for Collembola, a group in which all specimens are
identified to species, were recalculated based on biweekly density estimates.
Diversity in Test was consistently higher than in Control, but has decreased
gradually from year to year in both sites. Overall, despite the relative
intractability of the arthropod data base, we believe it important to

document the nature of the differences between sites, as well as their magnitude wherever possible.

With respect to earthworms, we deal with the surface-dwelling Dendrobaena octaedra, common to both sites; the intermediate Lumbricus rubellus, useful for year-to-year comparisons within Test; and the soil-dwelling Aporrectodea tuberculata (Test) which can, in some respects, be compared to A. turgida in Control.

The epigeic D. octaedra population has undergone wide annual fluctuations in Control, but reached all-time lows in both sites in 1989. Causes for these numerical changes lie in reproductive and growth parameters (see below). Lumbricus rubellus has remained relatively stable over all years, and A. tuberculata underwent slight overall increases since 1984.

2. For various population attributes other than abundance per se (many of which are directly or indirectly related to abundance), results can be summarized as follows:

a) Behavioral traits: Activity of arthropods (measured by numbers trapped) fluctuates in response to environmental factors as well as to internal, physiological stimuli. After repeated attempts to use temperature and relative humidity as explanatory variables for fluctuating captures, we conclude that the strength of this data base lies in tight correlation between seasonal catches in Test and Control. Such correlations bear witness to the fact that internal physiological clocks are synchronized between sites for a given species; and that modulation by temperature (which does not act linearly) is of similar magnitude in Test and Control.

Among Collembola, S. henshawi has shown significantly correlated activity patterns in Test and Control. Orchesella hexfasciata captures,

on the other hand, differed between sites in 1987 as well as 1988 for unknown
reasons, peak activity tending to occur 1 to 2 weeks earlier in Test than
in Control.

Among Acari, _Nanorchestes_ sp. is active year-round, with good correlation
between sites. The same can be said for _Trombidium auroraense_, a spring-
breeding velvet mite with activity maxima in May, and for several species
of carabid beetles, both spring- and summer-breeders. The reduced total num-
ber of Carabidae captured in 1988 in Control (1200 vs. approx. 2000 in Test,
which is consistent with previous years) provides the major discrepancy
observed so far.

Behavioral responses to litter and soil moisture are particularly
important determinants of earthworm vertical distribution. Periodic coloni-
zation of litter by _D. octaedra_ has been well correlated between sites in
all years. The proportion of _L. rubellus_ present in the litter layer,
regressed on litter moisture, gave significant results for 1984 to 1988,
but no relationship between these parameters seemed to exist in 1989. It
is possible that low proportions of small immatures (the most likely to
colonize litter) distorted these results. Vertical distribution of _A.
tuberculata_ is significantly related to moisture in the drier years:
neither 1984 nor 1989 produced significant regressions. By lumping several
years' data, we can show that _A. tuberculata_ and _A. turgida_ exhibit
essentially equal responses to soil moisture.

b) Population structure: for several taxa we have documented the
seasonal occurrence or frequency of life stages. They include _I. notabilis_,
surface-active as well as soil- and litter-dwelling mites (by size mea-
surements or identification of discrete developmental stages), and
earthworms (for which body mass and state of sexual development form

the data base).

Isotoma notabilis has so far been the most constant indicator species, peaks of recruitment and adult maturation coinciding in Test and Control. Among Acari, Asca aphidioides has undergone such drastic population decreases in both sites that the low number of specimens we obtain may endanger the future usefulness of the data base, by introducing variation through random chance.

Only for earthworms are 1989 data at hand. We can now document the seasonality of recruitment and growth for all relevant species. Reasons for the relative stability of D. octaedra numbers in Test lie in somewhat longer maturation times and concomitant greater longevity: in Control, large juveniles become reproductive the year after emergence, then probably die; in Test, a higher proportion of large immatures overwinter and contribute to the adult contingent of the second year after emergence. For L. rubellus, we estimate that approx. 1.5 years of growth are needed to reach adulthood. Neither in A. tuberculata nor A. turgida are growth patterns clearly discernible, because peak hatchling abundance can occur at any time of the season. Pulses of recruitment are then quickly lost in the pool of various-sized juveniles. We found some indication, however, that both species require at least two years to reach maturity, and may survive, and intermittently reproduce, for several years thereafter.

c) Reproductive parameters: we currently have data on carabid fecundity for two species and three years, but are rapidly increasing the data base to include more species and all years in which they are trapped in sufficient numbers. So far, between-site differences have not been significant; we believe these estimates may be important in the future.

Weights of earthworm cocoons remain a statistically tight parameter for all species. In several respects (though not all quantifiable), reproductive activity of A. tuberculata and A. turgida are comparable in terms of seasonal clitellate occurrence and resulting cocoon densities. Specific patterns of reproduction, and their potential variability, are now well quantified for all species of interest, including consistent differences between sites (D. octaedra), between years in Test (L. rubellus) and between species and sites (A. tuberculata and A. turgida).

3. System-level parameters:

Litter inputs were of approximately equal magnitude as in previous years in both sites. Turnover times for natural litter have proven more variable than anticipated. In Control, 1989, a turnover time of 1.37 years exceeded any previous estimate. We speculate that the low population of D. octaedra, a major litter decomposer, may be responsible. Decomposition studies (litterbags) raise the same possibility again: litterbags of the 1988-89 series retained significantly more litter mass in Control than in Test after one year in the field. First-year decomposition rates in Test (1986 vs. 1989) were essentially equal, i.e., remained stable. We have expanded this project element in order to obtain additional first-year decomposition data for each of the years to come.

I. ENVIRONMENTAL MONITORING

1. Precipitation

Monthly rainfall totals were again below average, with the notable exception of June (Table 1). Most of June rains occurred within a brief period of one to two weeks, followed by drought (Fig. 1). Yearly totals were higher in Test than in Control, due mainly to more ample precipitation during August (Table 1).

Table 1. Monthly precipitation totals (mm) in Test and Control, 1989, and 30-year means (Crystal Falls Weather Station) for the area at large.

	May	June	July	Aug	Sept	Oct	Total
Control	54.4	133.2	47.4	32.0	62.7	31.8*	361.5
Test	64.2	141.9	44.3	74.3	73.2	35.7*	433.6
30-year mean	81.0	105.4	91.4	98.5	84.6	52.8	513.7

*) Readings to October 23

2. Litter and soil moisture

Biweekly litter moisture fluctuations (Fig. 2) were highly correlated between sites, much as in the past. Moisture levels of A and B horizons were somewhat more variable than in previous years, Test A horizon moisture being more stable than Control (Fig. 2).

3. Temperature

Soil and air temperature readings by means of Omnidata equipment
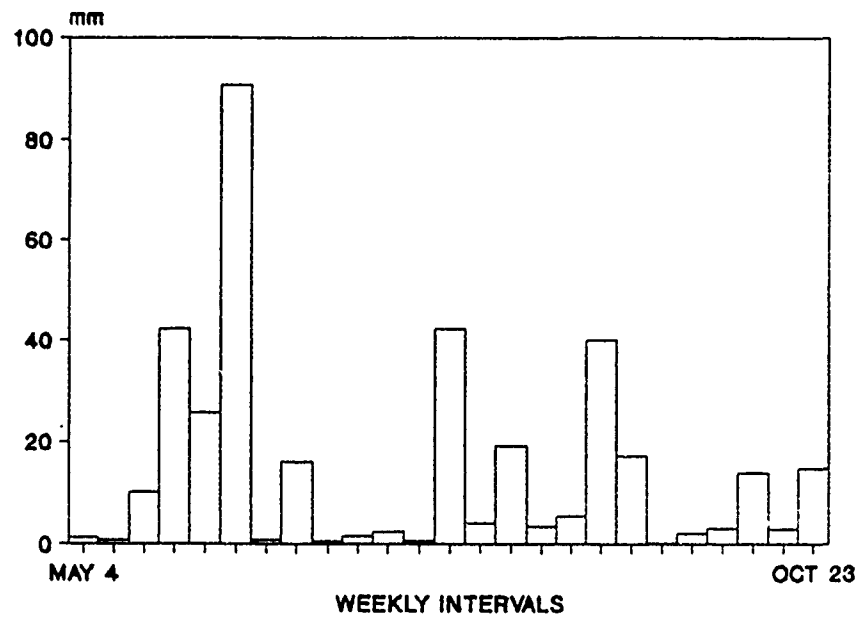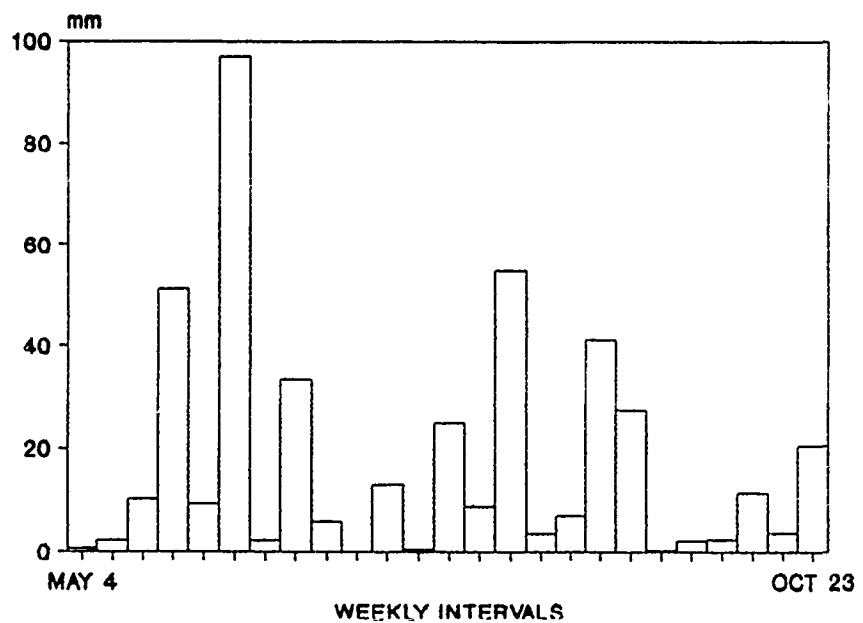
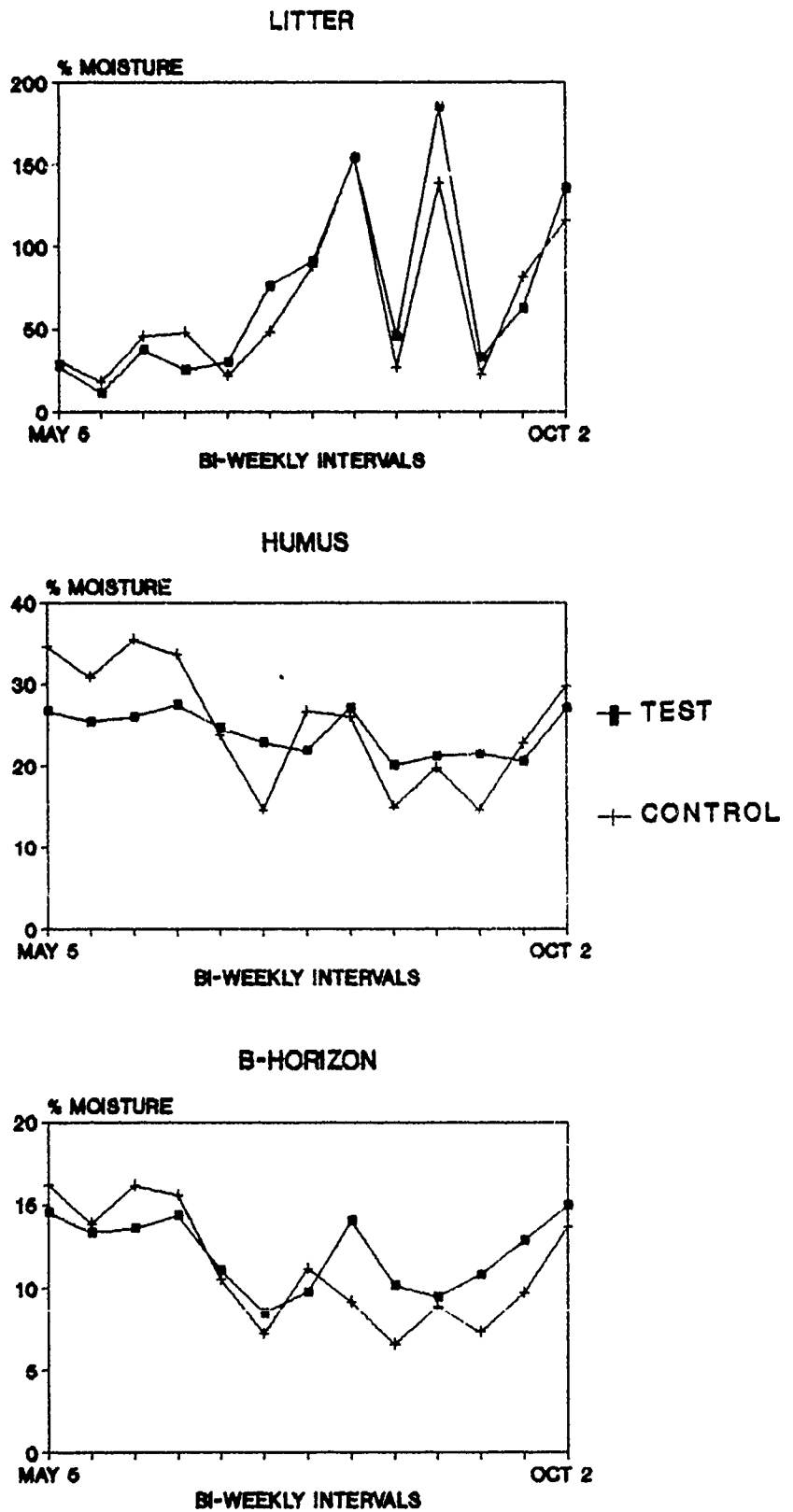Fig. 1. Weekly precipitation totals (mm), Test and Control, 1989.

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Fig. 2. Biweekly estimates of substrate moisture (%), 1989.

were successful; summaries are available for faunal analyses if needed. Air temperatures in 1989 were unusual only in that daily maxima during the week of October 23 were consistently above 20°C, daily means during that time ranging from 11 to 16°C.

II. SOIL AND LITTER ARTHROPODA

Data for the 1988 season are available for this report. Samples of the 1989 season have been sorted, but species identifications are not yet complete.

1. Collembola

1.1. Diversity

Shannon-Wiener diversity indices were re-calculated for the years 1986-1988 (years in which sugar floatation was performed), yearly averages thus being based on diversities per sampling date. Anova of these seasonal estimates showed both site and year effects to be significant. This is not surprising, since diversity indices for Test Collembola have been consistently higher than for Control, and indices have been decreasing in both sites from 1986 to 1988 (Table 2). Much higher densities of Onychiuridae in Control may well be responsible for lower overall diversity in that site.

Table 2. Average (± SD) yearly diversity indices (Shannon-Wiener) for Test and Control collembolan communities, 1986-1988.

	1986	1987	1988
Test	2.35 ± 0.21	2.25 ± 0.28	2.03 ± 0.36
Control	2.01 ± 0.23	1.67 ± 0.17	1.47 ± 0.16

1.2. Abundance

As point of reference and for the sake of periodically including a complete set of data, Table 3 lists mean annual densities for all

species so far encountered in Test and Control. NOTE that 1984 and 1985 data do not include numbers recovered by sugar floataion, which was begun in 1986. Based on preliminary examination of extraction data, we tentatively assume that extraction efficiency is comparable for Test and Control soil cores. The main affected taxa are Onychiuridae and, even more so, Hypogastruridae. In the latter, 70-80% of individuals from both sites are recovered by floatation.

We can now examine selected taxa listed in Table 3. Whether at the species or family level, major year-to-year fluctuations (by a factor of 2 or more) appear to be the rule for many taxa. Some of the rare species (e.g., the isotomids Anurophorus septentrionalis and A. altus) are occasionally encountered in large numbers in single samples, so that they appear common in one year, but totally absent in others.

If we search for site similarities, excellent correlations are obtained for Isotoma notabilis densities (r= 0.98; Fig. 3) as well as for total Isotomidae (r= 0.95). Total Hypogastruridae and their dominant member, Willemia intermedia (r= 0.95 and 0.99 respectively) also showed comparable trends in Test and Control. Assuming that efficiencies are indeed similar between sites (detailed analysis in progress), then these are valid trends which can be used for future site comparison. In fact, Collembolan densities as a whole (both with and without Onychiuridae) are highly correlated between sites (r= 0.99).

1.3. Seasonal densities

Most soil-dwelling Collembola, dominated by Onychiuridae, are so strongly aggregated that analysis of seasonal fluctuations becomes meaningless. Among litter-dwellers, the sminthurid S. henshawi showed signi-

Table 3. Mean annual abundance/ m^2 (litter + soil estimates summed) of Collembola, 1984-1988.

NOTE: Onychiuridae 1984 and 1985 not corrected for extraction efficiency.

	1984		1985		1986		1987		1988	
	T	C	T	C	T	C	T	C	T	C
S. henshawi	153	186	259	301	224	357	198	321	356	325
S. lepus	8	1	18	8	13	9	54	27	40	23
S. macgillivrayi	8	1	28	2	12	4	8	10	10	3
A. amarus	31	20	22	25	12	5	72	4	6	35
A. benitus	68	23	146	146	34	58	40	0.2	16	1
A. caecus	–	–	–	–	0.1	35	42	35	17	4
D. aurata	–	16	–	13	–	9	4	19	4	8
D. marmorata	–	–	–	0.2	–	1	–	55	4	1
B. russata	–	–	–	–	4	–	–	–	–	–
S. intermedius	–	0.1	–	–	–	12	–	–	–	–
B. atra	–	–	–	–	–	0.1	–	–	–	–
B. hortensis	–	0.1	–	–	–	–	–	–	–	–
S. quadrimaculatus	–	0.1	–	–	–	–	–	–	–	–
SMINTHURIDAE Σ	268	247	473	495	299	490	418	471	453	400
I. notabilis	1141	1628	1684	2940	1782	2690	2220	3739	1076	1542
I. minor	242	375	158	143	408	292	304	541	137	200
F. bisetosa	–	4	162	259	171	193	94	280	34	114
F. nivalis	9	223	90	194	97	178	232	179	80	127
A. binoculatus	30	77	41	88	23	101	31	152	18	41
I. nigrifrons	90	55	91	21	18	32	67	0.4	5	25
P. minima	15	49	11	90	12	5	43	44	14	90
I. viridis	5	–	19	0.1	75	0.1	66	–	30	0.1
A. septentrionalis	–	315	–	–	–	66	–	46	6	24
A. altus	–	–	–	186	–	14	–	–	–	–
I. nympha	–	0.5	–	–	–	0.5	–	–	–	–
I. pseudocinerea	–	–	3	–	–	–	–	–	–	–
C. exilis	–	–	4	15	–	–	42	15	–	12
C. decemoculatus	5	–	–	–	–	–	–	–	–	–
ISOTOMIDAE Σ	1537	2727	2263	3936	2586	3571	3099	4996	1400	2175

Table 3 continued:

	1984 T	1984 C	1985 T	1985 C	1986 T	1986 C	1987 T	1987 C	1988 T	1988 C
T. flavescens	219	34	440	51	495	59	876	24	277	142
O. hexfasciata	107	50	195	39	234	28	528	73	170	95
E. comparata	19	80	37	82	84	34	128	58	72	222
P. violenta	353	–	278	–	395	8	722	0.1	357	–
E. nivalis	23	5	40	4	69	8	19	4	40	8
W. buski	0.3	17	12	4	46	12	74	29	30	39
T. lamelliferus	89	16	45	1	73	4	182	–	49	–
L. violaceous	4	4	12	–	8	0.3	18	1	21	–
L. helenae	3	11	5	7	4	8	8	1	10	8
L. lignorum	0.4	–	0.1	–	4	–	4	–	9	–
E. purpurascens	4	1	0.5	0.5	4	12	4	23	0.1	–
ENTOMOBRYIDAE Σ	822	218	1065	189	1416	173	2563	213	1035	514
W. intermedia	–	–	58	86	236	270	428	616	221	287
W. similis	–	–	77	0.4	162	69	281	227	183	237
A. furcifera	–	–	38	112	50	212	35	466	38	134
N. muscorum	14	24	3	107	48	26	71	165	69	16
A. pygmaea	0.3	8	–	8	12	100	58	155	25	42
F. sublimis	–	–	0.1	25	–	24	–	8	–	0.5
P. saxatilis	10	51	–	5	4	8	–	0.1	–	–
X. acauda	4	86	–	0.1	0.3	–	–	–	0.4	0.1
X. pallescens	–	–	1	8	–	5	0.5	–	3	6
P. aureofasciata	–	–	4	5	–	12	0.1	4	–	0.1
P. caeca	–	–	–	8	1	–	–	1	–	5
O. substriata	–	–	–	23	–	–	–	–	–	–
W. denisi	–	–	–	–	8	–	–	–	–	–
M. spirillifera	–	–	–	–	8	–	–	–	–	–
A. granaria	4	85	–	–	–	0.1	–	–	–	–
X. christianseni	–	–	–	–	–	0.1	–	–	–	–
N. barberi	–	–	–	–	–	0.1	–	–	–	–
P. indiana	–	0.2	–	–	–	–	–	–	–	–
HYPOGASTRURIDAE Σ	32	254	213	387	529	726	874	1642	539	728

Table 3 continued:

	1984 T	1984 C	1985 T	1985 C	1986 T	1986 C	1987 T	1987 C	1988 T	1988 C
N. minimus	-	-	145	137	11	270	80	259	13	111
N. minutus	0.3	17	8	4	-	15	1	18	-	5
N. tristari	257	208	4	0.2	8	2	-	0.2	-	-
N. snideri	-	-	0.2	36	-	6	-	4	-	-
NEELIDAE Σ	257	225	157	177	19	293	81	281	13	116
T. mala	1080	5850	1708	4382	2343	17870	4554	24347	4055	17138
T. granulata	1332	1421	1286	1459	3551	5658	5563	11254	4547	7721
T. iowensis	-	-	359	193	1554	589	2449	4188	2356	2767
T. clavata	104	196	246	204	746	673	723	1989	321	742
T. yosii	288	195	100	47	25	169	78	631	18	112
T. falca	-	-	81	-	42	-	208	-	371	13
O. similis	50	188	35	112	269	596	165	916	183	321
O. encarpatus	-	34	-	-	-	1	8	-	38	-
O. affinis	-	-	-	29	8	47	4	88	-	45
O. armatus	-	-	-	-	-	-	4	-	-	-
T. talus	-	-	-	-	-	-	-	12	-	-
T. hades	-	-	4	-	-	-	-	-	4	-
O. parvicornis	-	-	-	16	-	-	-	-	-	-
ONYCHIURIDAE Σ	2854	7884	3819	6442	8538	25603	13756	43425	11893	28860
TOTAL (- Onychiuridae)	2916	3671	4171	5184	4849	5253	7035	7603	3440	3933
TOTAL (+ Onychiuridae)	5770	11555	7990	11626	13387	30856	20791	51028	15333	32793
TOTAL N SPECIES	36	41	46	37	46	55	46	47	45	44
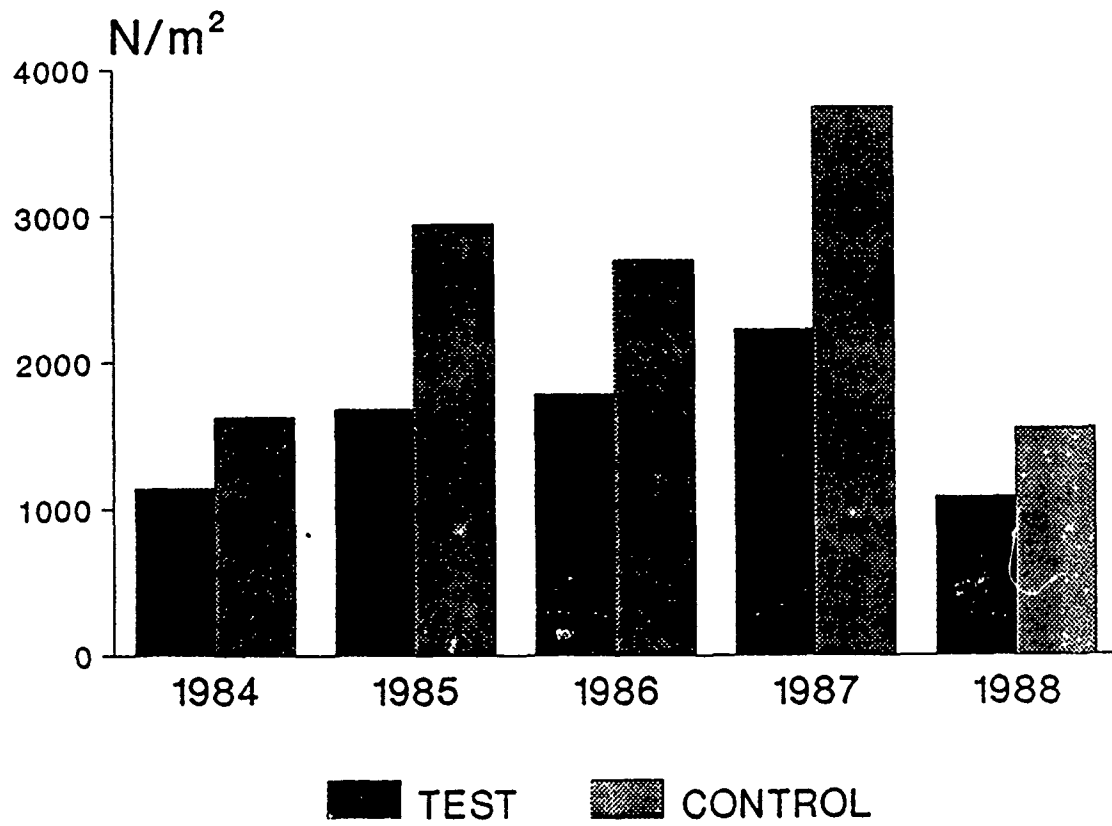
Fig. 3. Mean annual densities (litter + soil summed) of Isotoma notabilis in Test and Control, 1984-1988.

ficantly correlated density fluctuations in 1988 (r= υ.71, P<0.01).

For $\underline{I}$. $\underline{notabilis}$, which frequents both litter and soil, correlations were even better (r_{litter}= 0.84, r_{soil}= 0.82, P<0.001). In this species, none of the developmental stages we recognize seems to have a "vertical preference", i.e., distribution seems to be independent of seasonal population structure. Clearly, however, the proportion of the population present in leaf litter depends on litter moisture: at < 20% moisture, virtually no individuals are found in litter; at higher moisture levels, movement into the upper stratum is somewhat variable, and may be modulated by temperature (non-linearly). Moisture alone explains about 40-50% of observed variation.

1.4. Population structure of Isotoma notabilis

We have shown earlier that division of the population into developmental classes yielded seasonal frequency distributions with significant general association between sites. These data are based on size measurements of all individuals extracted from leaf litter and soil, permitting assignment to instar I, other juvenile, and adult classes. Measureme · of 1988 specimens is still in progress, but we can discuss 1987 data in perspective to earlier years.

Mean annual density estimates for 1987 were consistent with previous years in terms of the proportionality of hatchlings : juveniles : adults (Fig. 4). The early May peak of hatchlings observed in 1984 through 1986 was much reduced in 1987 (Fig. 5). The mild winter of 1986-87, and relatively high temperatures in the first week of May apparently contributed to an early molt of hatchlings into subsequent juvenile
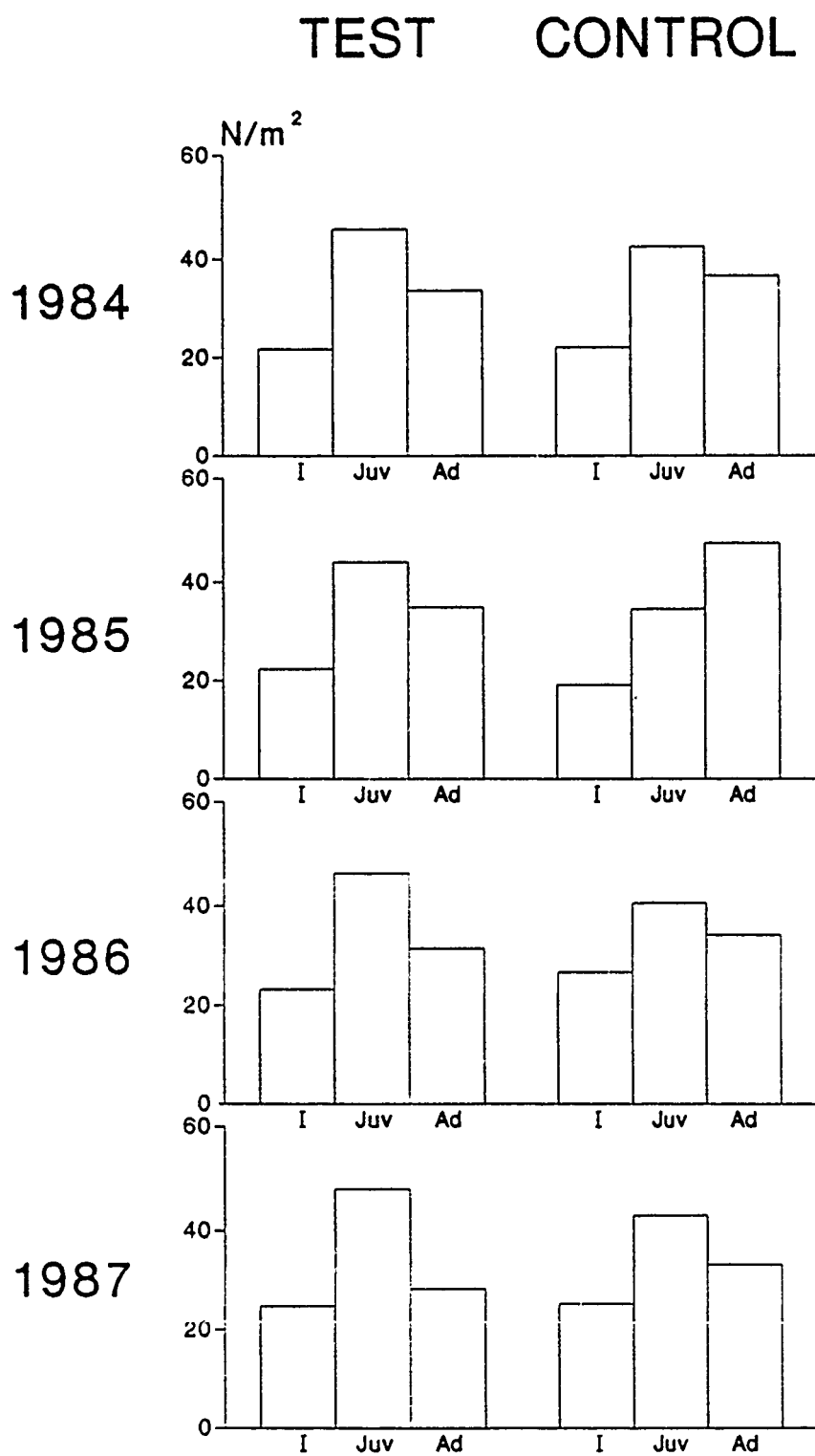
TEST CONTROL

Fig. 4. Mean annual densities of instars I, other juveniles and adults of _Isotoma_ _notabilis_.
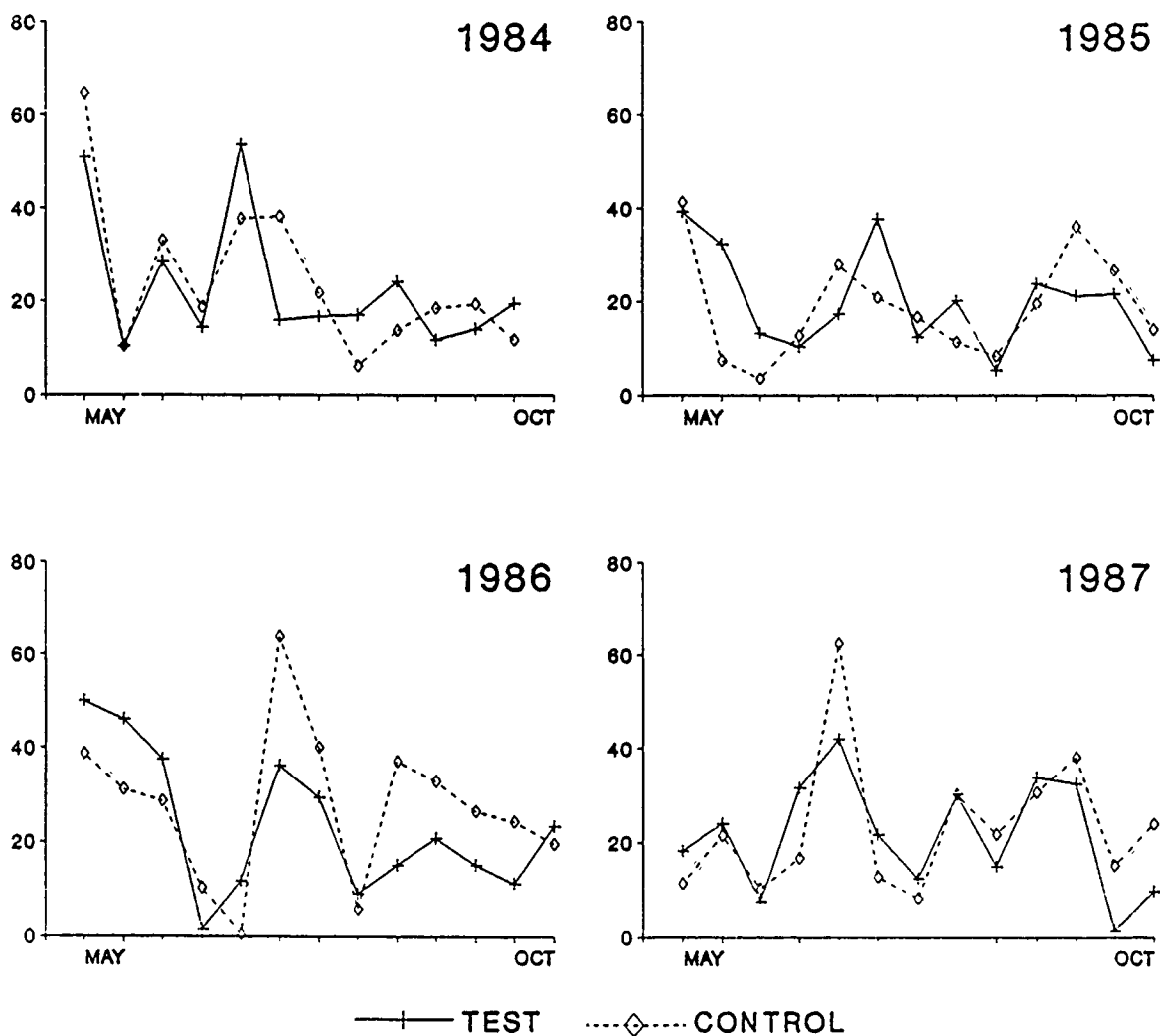
Fig. 5. Seasonal frequencies of instar I (in % of total population) of *Isotoma notabilis* in Test and Control.

stages (the latter constituting 60% of the total population in both

sites on May 5). Recruitment peaks in June-July and, less distinctly, in September, were similar to previous years' patterns (Fig. 5), and were synchronous in Test and Control (r= 0.73, P<0.01). Considering that the animals remain in instar I for only a few days, point-sampling at two-week intervals increases the chances of missing their peak occurrences in either or both sites. We submit that lower levels of significance should also be acceptable, and should provide sufficient evidence that the populations reproduce and develop at equal rates in both sites.

Anova of seasonal frequency distributions (without replication) of adults and hatchlings confirmed our conclusions: neither site nor year effects were significant ($P >$ or $\gg 0.2$); as would be expected, class effects, class x date and class x date x year effects were significant ($P <$ or $\leqslant 0.01$); most importantly, no interactions involving sites were significant.

2. Acari

2.1. Density

Yearly mean abundance of the three taxa we monitor in detail have fluctuated synchronously in Test and Control (Fig. 6), correlation coefficients ranging from 0.89 (Mesostigmata A, P<0.05) to 0.98 (Asca aphidioides, P<0.01). Asca aphidioides has remained at very low average densities since 1986; the other two species underwent varying increases or reductions, parallel in Test and Control (Fig. 6).

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Mesostigmata sp. A

Asca aphidioides

Nanorchestes sp. A
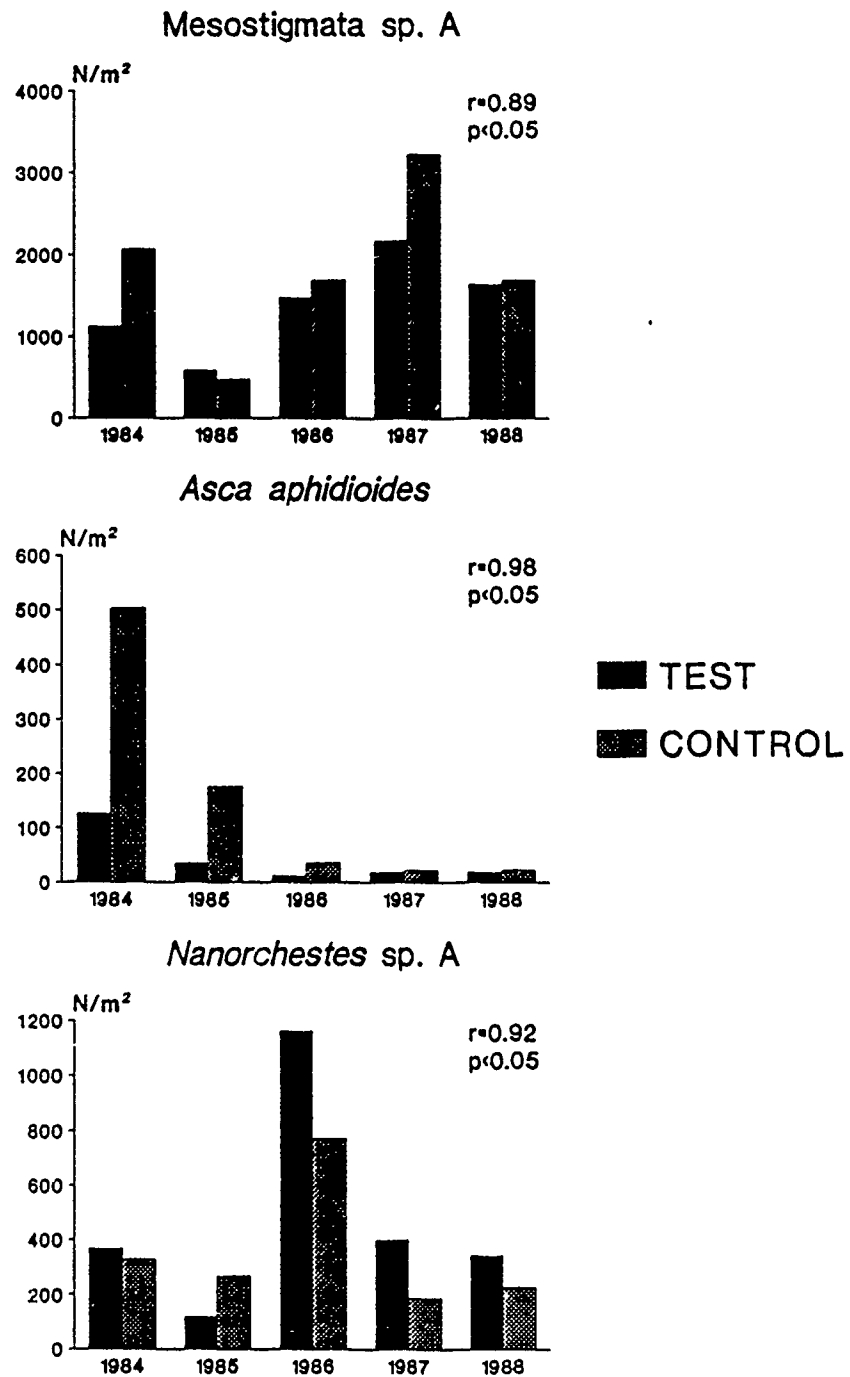
Fig. 6. Mean annual densities of selected Acari, 1984-1988.

2.2. Seasonal stage structure

Because of relatively low numbers of A. aphidioides and Mesostigmata A
obtained from soil and litter, seasonal frequencies of developmental
stages were summarized by month (Figs. 7-11 show data for A. aphidioides).
Anova of these distributions (leaving out deutonymphs because of the
interdependency of percentiles) allows the following conclusions for
both species: seasonal population structure does not differ between
sites but does vary between years, obviously as a result of temperature-
dependent development rates; site x year interactions have so far not
been significant, indicating that year-specific differences have
occurred in parallel in Test and Control.

For A. aphidioides in particular, severely reduced populations in both
sites (Fig. 6) are beginning to endanger this data base. Test and Control
data for 1988 (Fig. 11), as compared to, for instance, 1984 (Fig. 7) are
marginally correlated. With numbers of specimens as low as they now are,
we suspect that random chance may render the data useless for project
purposes.

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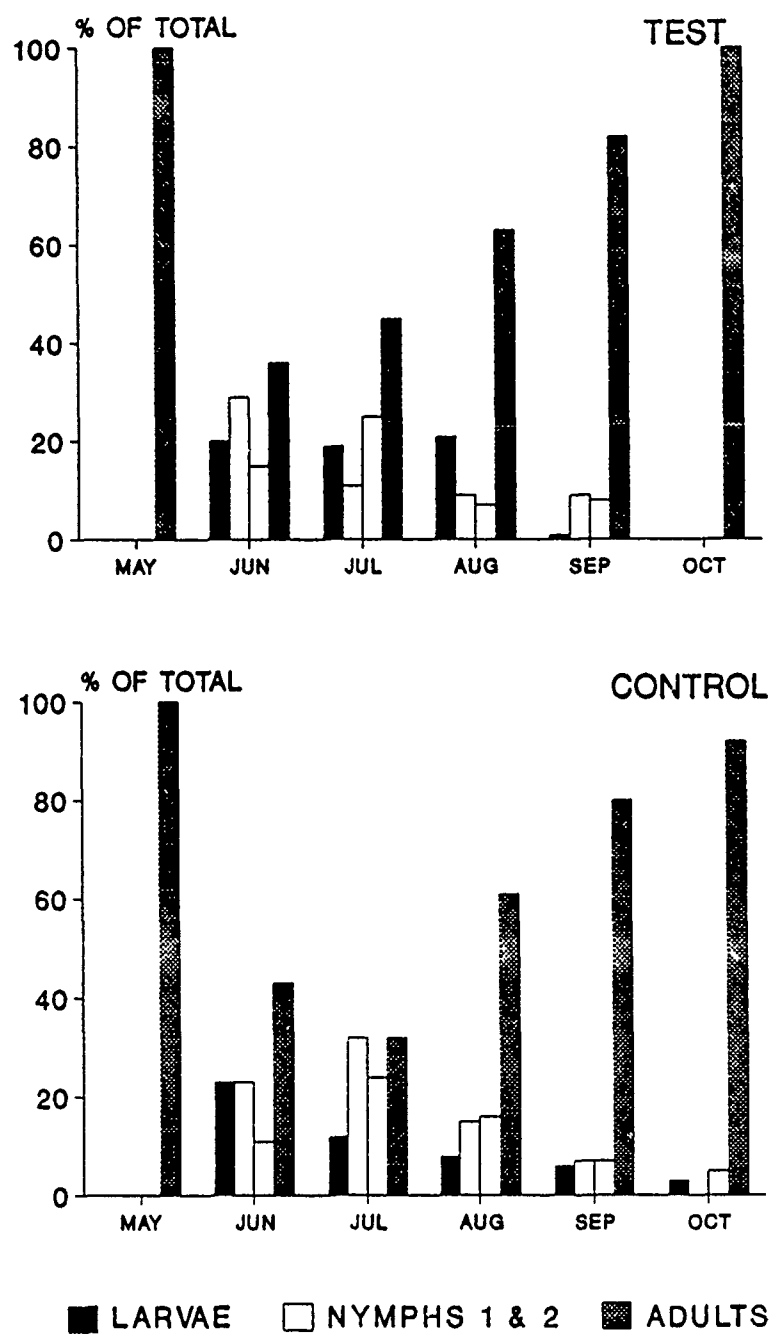
Fig. 7. Population structure of Asca aphidioides , 1984.
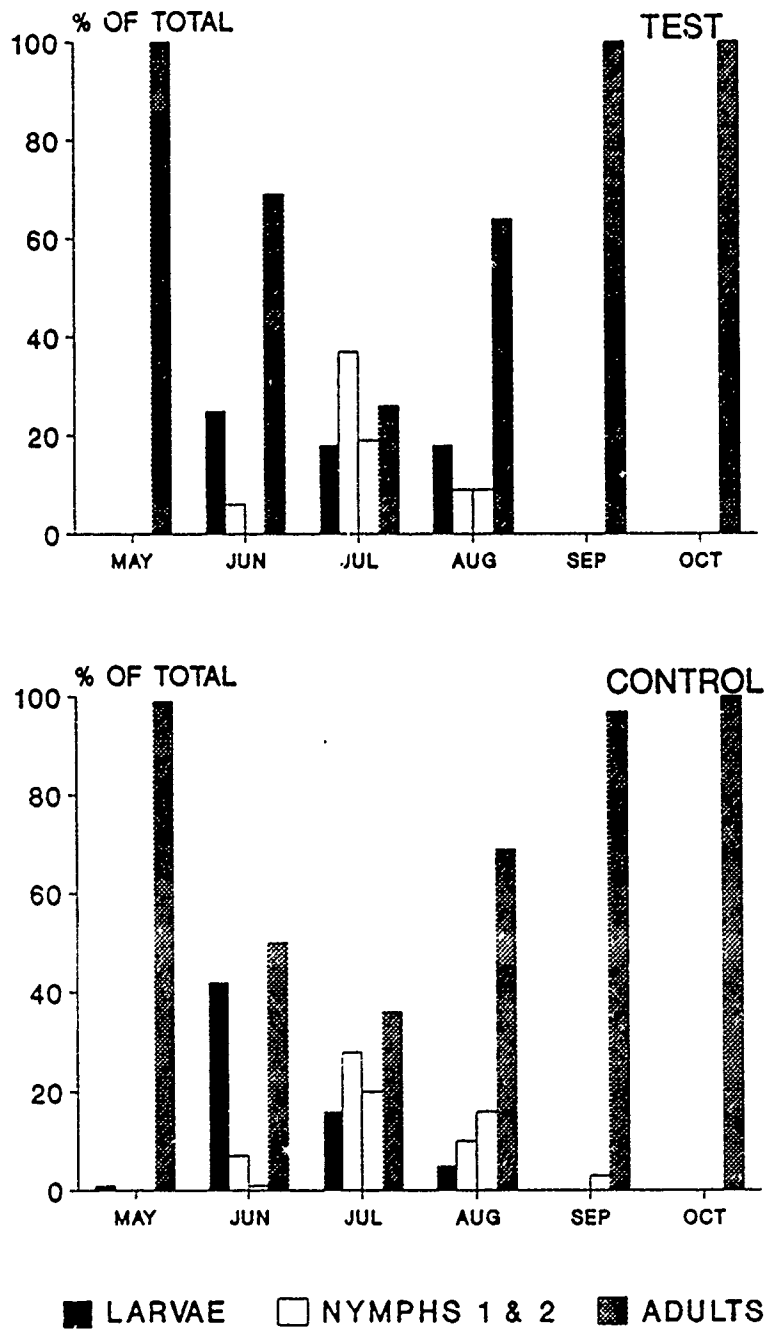
Fig. 8. Population structure of <u>Asca</u> <u>aphidioides</u>, 1985.
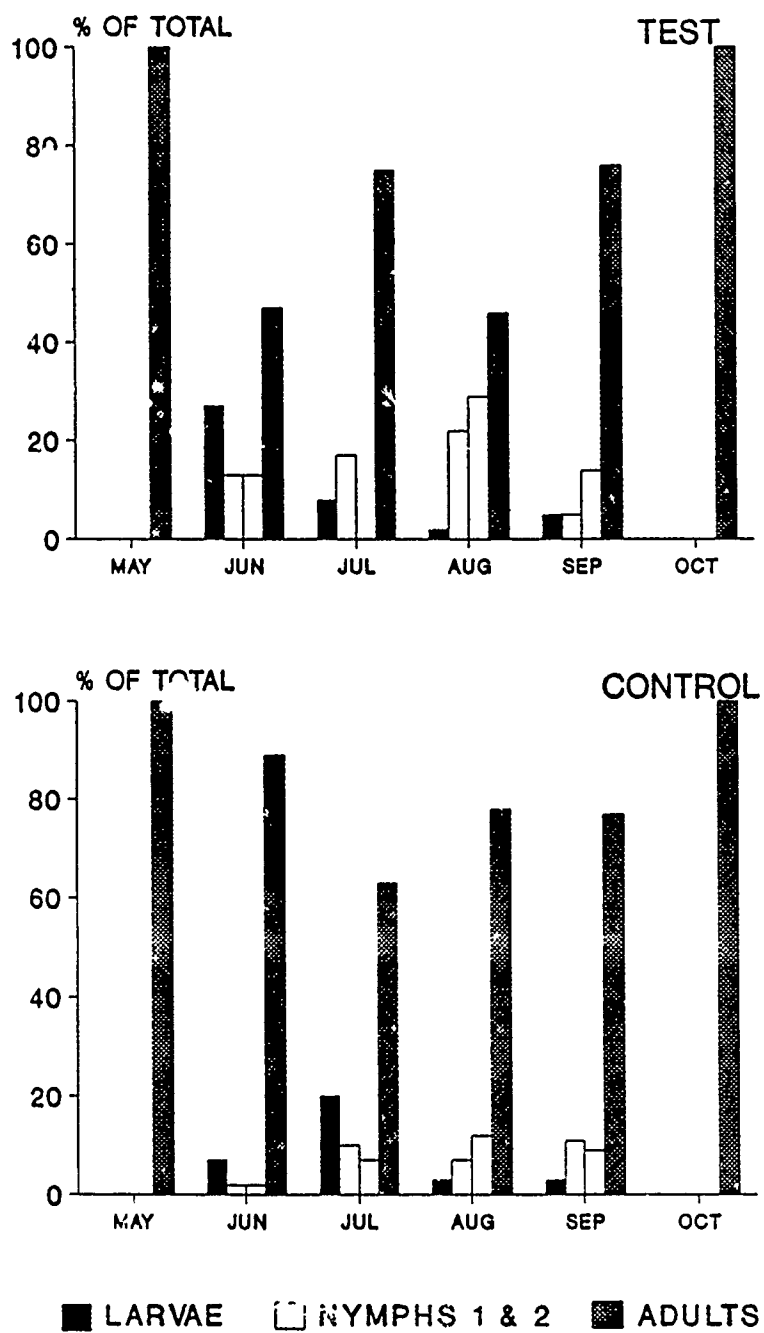
Fig. 9. Population structure of <u>Asca aphidioides</u>, 1986.
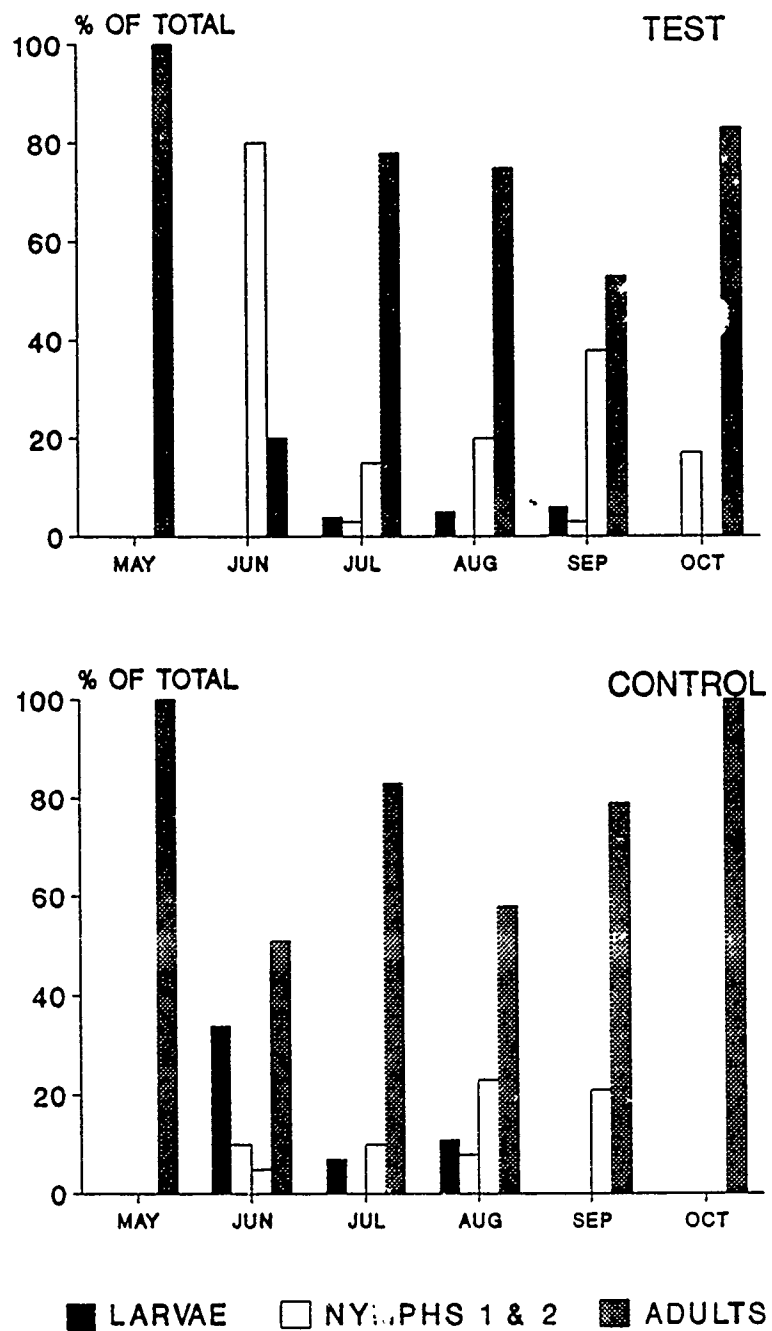
Fig. 10. Population structure of Asca aphidioides, 1987.
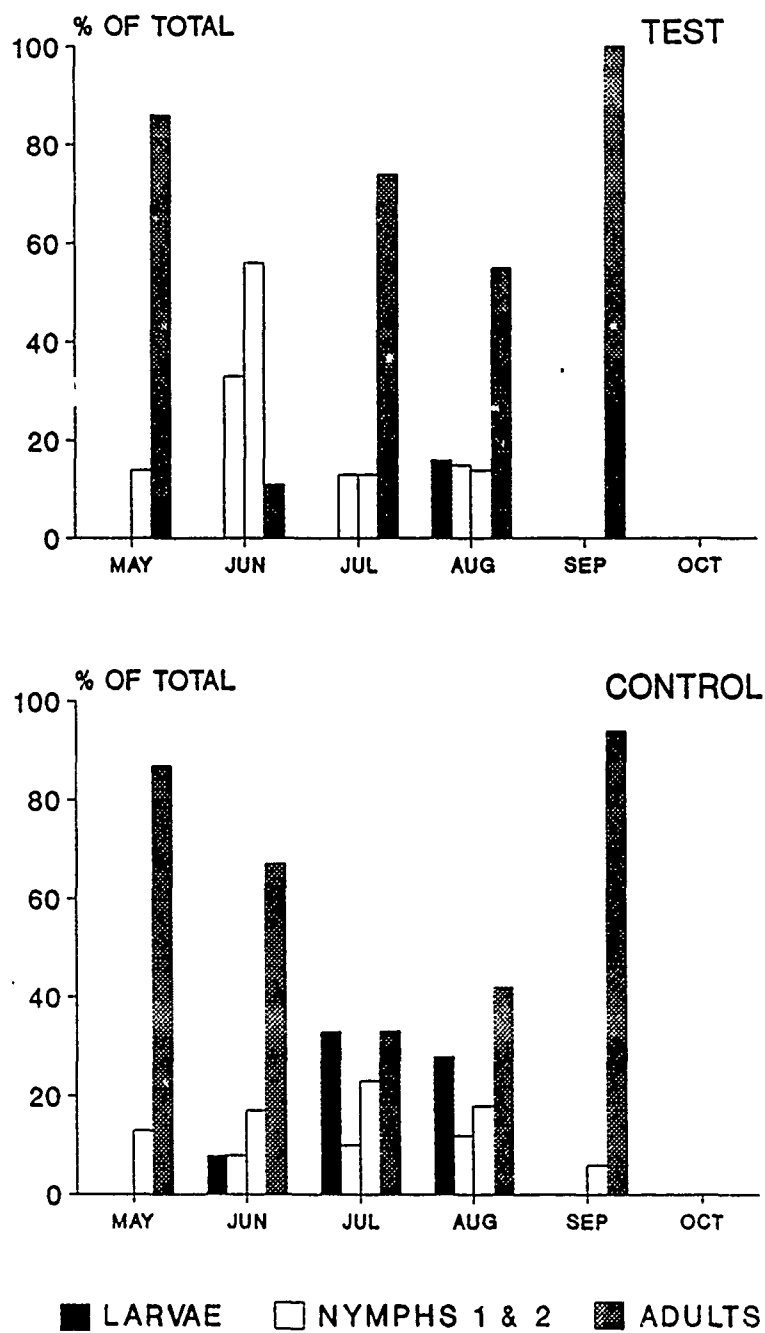
Fig. 11. Population structure of <u>Asca</u> <u>aphidioides</u>, 1988.

III. SURFACE-ACTIVE ARTHROPODA

Data for 1988 are available for analysis. Rough-sorting and concurrent species identification of 1989 samples are well under way, but not yet complete.

With the single exception of Nanorchestes A in Test, relationships between seasonal population densities and trap catches could not be demonstrated. Frequently, species of low abundance are captured in traps in relatively large numbers. Indeed, the purpose of trapping is to obtain data other than abundance estimates, and data on activity of species for which abundance cannot be estimated at all (e.g., Carabidae).

After repeated attempts to use temperature and relative humidity as explanatory variables for seasonally fluctuating trap catches, we have come to the following conclusions:

a) Although the data show a general, and occasionally obvious, relationship between activity and environmental variables, rarely can more than 50% of variation be explained by them. "Season" and "seasonal temperature" have biological meaning which differs from their statistical interpretation. Example: Sminthurinus henshawi catches (Fig. 12) peaked on date 4 (May 30-31), the first date of the year when mean day and night temperatures exceeded 19°C and minima exceeded 15°C. On date 5, as well as later in the season, identical temperature and RH combinations never again produced comparable activity peaks (Fig. 12).

b) For species with restricted times of activity, it is difficult and arbitrary to delimit "maximum activity periods" and to quantify ambient effects on activity fluctuations within them. Velvet mites and carabids in particular are active in response to physiological stimuli; if patterns

of reproduction and development are equal in Test and Control, then seasonal occurrence and relative frequency in pit-traps should be correlated between sites.

c) Therefore, for all species, significant correlation between trap catches in Test and Control provides valid evidence upon which to judge potential disturbance.

1. Collembola

1.1. Numbers trapped

Table 4 furnishes basic information on total numbers captured, of species numerous in either site, and on family-level and overall totals. We may simply point out that vast year-to-year variation appears to be the rule for most species. Orchesella hexfasciata and Entomobrya comparata in Control, for instance, reached maxima in 1988 which were mainly responsible for entomobryids outnumbering sminthurids for the first time in that site. At the family level, dominance relationships can be strongly influenced by single species' activity, for instance Hypogastruridae by Pseudachorutes saxatilis in 1985. These data offer l' tle means of site comparison, other than to document the potential range of variation in both sites. Approximately 75-85% of species are shared between sites, variable mainly due to occurrence of rare taxa in either site. While Tomocerus flavescens reaches high counts only in Test, and Lepidocyrtus paradoxus only in Control, Sminthurinus henshawi, Sminthurides lepus and O. hexfasciata have been the dominant or near-dominant Sminthuridae and Entomobryidae in both sites in most years (Table 4).

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Table 4. Total annual pit-trap catches of selected taxa of Collembola in Test and Control.

	TEST				CONTROL			
	1985	1986	1987	1988	1985	1986	1987	1988
S. henshawi	1637	1435	1992	2811	2606	2934	4123	5084
S. lepus	669	236	1049	503	397	375	1019	824
D. aurata	5	0	4	1	468	976	2198	448
SMINTHURIDAE Σ	2423	1709	3124	3398	3593	4379	7607	6770
T. flavescens	4213	1965	2429	1684	842	242	280	165
O. hexfasciata	3201	3402	4137	3426	1099	421	1180	3549
E. comparata	35	80	119	150	287	87	157	1493
E. nivalis	531	1057	294	291	4	14	34	77
L. paradoxus	22	6	37	123	1142	961	2701	2649
P. violenta	166	381	606	916	1	1	47	0
ENTOMOBRYIDAE Σ	8433	7238	8209	7186	3479	1752	4495	8100
P. saxatilis	13	0	5	0	1925	198	348	379
HYPOGASTRURIDAE Σ	80	90	191	196	2122	292	456	463
ISOTOMIDAE Σ	582	513	486	292	751	392	562	188
TOTAL ALL SPP.	11518	9550	12010	11072	9946	6815	13120	15522
TOTAL N SPP.	36	29	32	33	30	28	32	33

1.2. Seasonal activity patterns

Strongly influenced by the dominant species, Entomobryidae tend to
be most active in early to late summer, while Sminthuridae exhibit some
distinct peaks in the spring and occasionally in the fall. We single out
only two species for discussion, S. henshawi and O. hexfasciata, in order
to illustrate potential similarities and discrepancies between sites.

In 1985, O. hexfasciata activity (Fig. 13) was strongly correlated
between sites (r= 0.92). In subsequent years, shifts in activity were ob-
served: in 1987 (r= 0.27) and 1988 (r= 0.65) in particular, peak captures
tended to occur 1 to 2 weeks earlier in Test than in Control. In 1988,
numbers caught were higher in Test prior to July 12, consistently lower
than in Control on all dates thereafter. We have no explanation at this
time, but will double-check the specimen and data bases to confirm the
validity of these observations.

Activity of S. henshawi, on the other hand, has shown reliable syn-
chronicity between sites (Fig. 12 illustrates 1988 data as example).
Striking activity peaks in May and June were observed in previous years as
well. As discussed above, these are caused by the first warming temperatures
(= extreme day-to-day temperature fluctuations of spring); the species
then settles into activity fluctuations of much smaller amplitude.

2. Acari

2.1. Trombidium auroraense

Brief, early-season activity of ovipositing adults is characteristic
of this species. Test and Control catches have been consistently and signi-
ficantly correlated (Fig. 14), the species being al·ost entirely day-active.
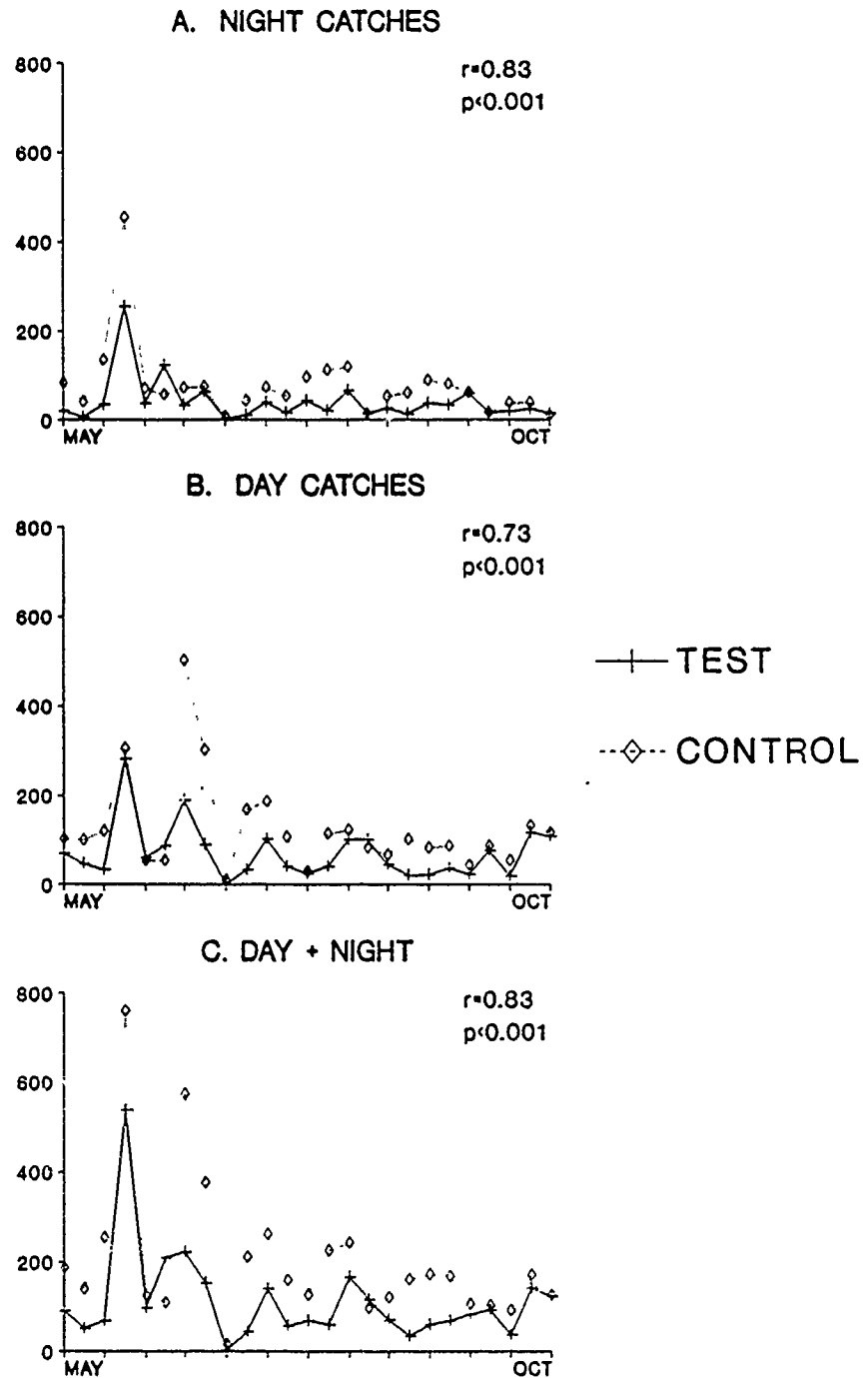
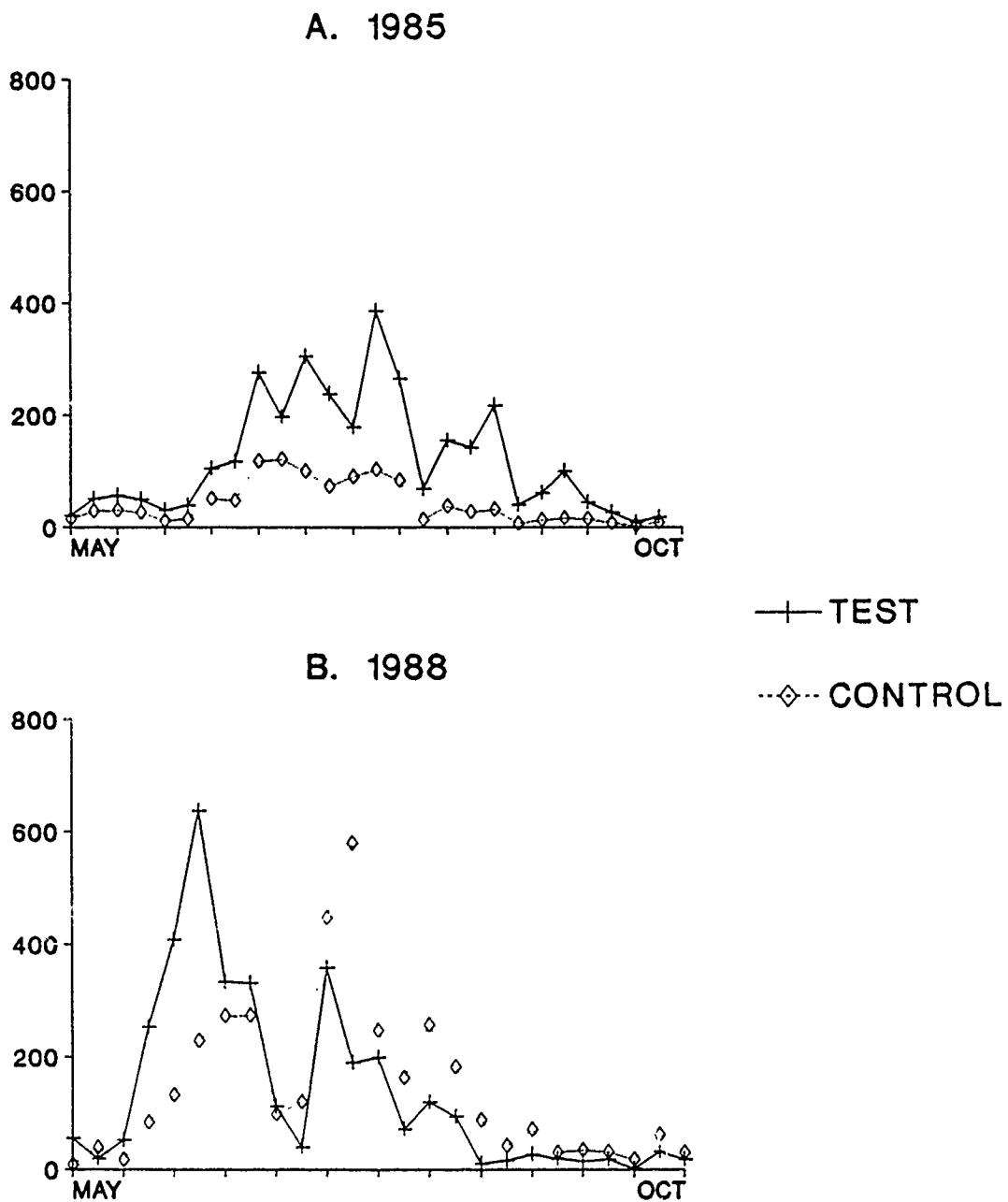Fig. 12. Total catches/ date (weekly intervals) of Sminthurinus henshawi in 1988, Test and Control.

A. 1985

B. 1988

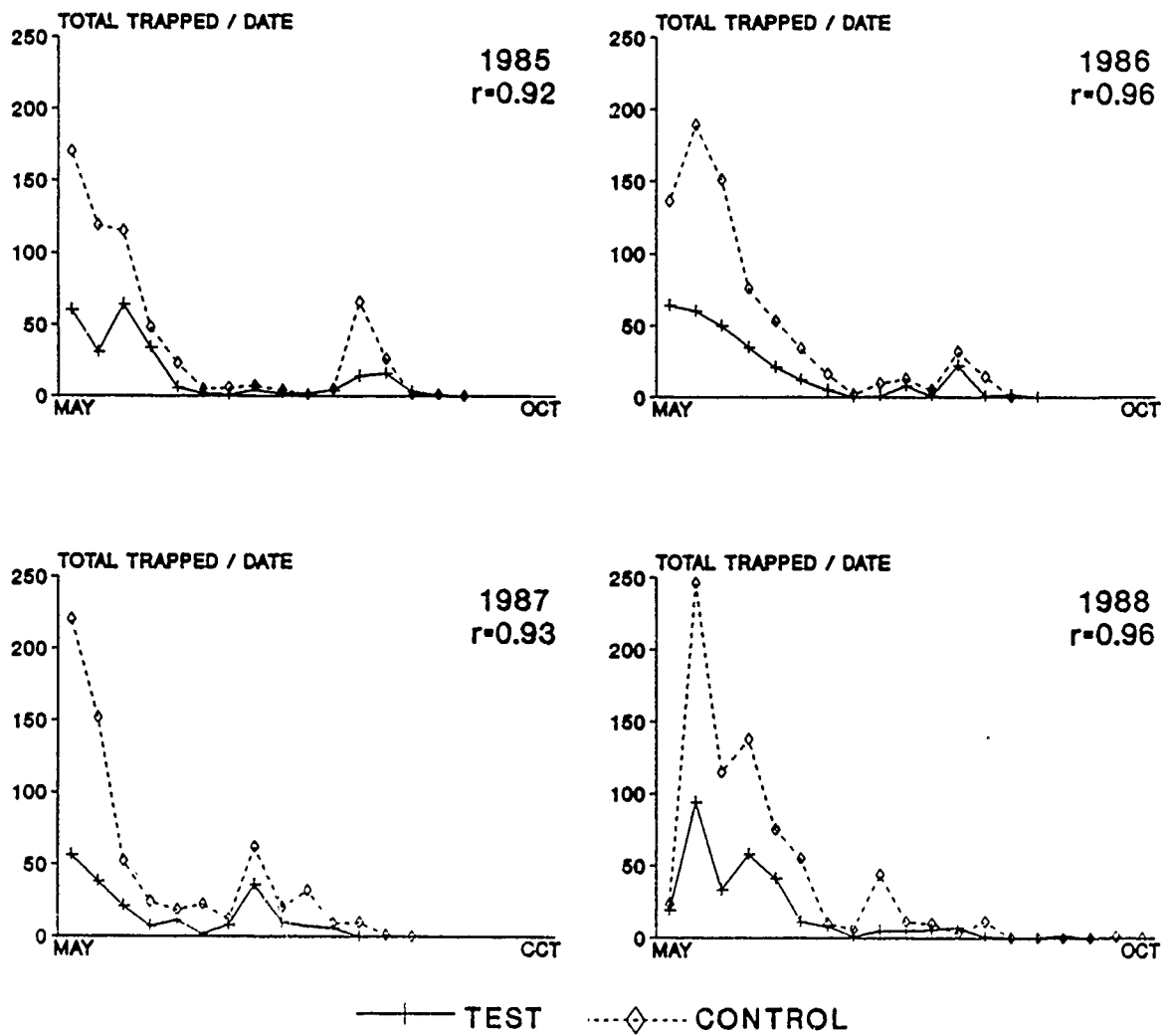Fig. 13. Total weekly catches of Orchesella hexfasciata in Test and Control, 1985 and 1988 opposed.

Fig. 14. Total weekly catches of Trombidium auroraense in Test and
Control, 1985-1988.

A second period of activity in mid- to late summer is attributable to larvae in search of insect hosts.

Adult activity is modulated by air temperature: in 1988, for instance, low catches on the first and third trap dates (Fig. 14) were related to average air temperatures of $< 10°C$. Overall, however, the physiological state of the animals is the overriding factor responsible for their seasonal activity patterns.

2.2. Abrolophus spp.

We have earlier reported tightly correlated mid-summer activity peaks for Abrolophus in Test and Control. Faintly in 1987, and clearly in 1988, Test data showed an unexpected May-June peak not mirrored in Control.

We are currently re-examining specimens from all years in order to determine how many species we are actually dealing with. There are r named species of Abrolophus for North America, and even distinction between deutonymphs and adults can be tenuous. With Dr. Calvin Melbourn's help, we are tentatively separating two species (in both sites), but will need to rear them in order to correlate different adult forms with their respective immature stages.

2.3. Nanorchestes spp.

Three taxa occur in our sites, of which one, Nanorchestes A, is most numerous. In this species, a tenuous relationship between mean annual population density and numbers trapped has emerged, but only in Test (r= 0.98; Table 5). Since this is not parallelled in Control, and is based on only four years' data, the relationship may well turn out to be spurious.

Table 5. Annual mean density (litter + soil) and total number of

Nanorchestes A trapped per year, 1985-1988.

	1985	1986	1987	1988
TEST				
N/m^2	119	1163	401	344
N trapped	1322	4405	1746	2216
CONTROL				
N/m^2	266	771	186	227
N trapped	808	5926	2329	4984

Nanorchestes spp. act much like Collembola, being active year-round.
Seasonal activity patterns were significantly correlated between sites
in all years (P $<$ 0.01 or better), coefficients ranging from 0.71 in 1987
to 0.92 in 1988 (Fig. 15).

3. Carabidae

3.1. Annual fluctuations in numbers

Serving as a periodic update, total numbers captured of each species
are listed in Table 6. Overall 1988 totals in Test remained high, but
were reduced to approximately 1200 in Control. Several species contributed
to this decline, Pterostichus adstrictus and Synuchus impunctatus in par-
ticular. In Test, P. melanarius regained its dominance, which had declined
in 1987 (Table 6).

We have retained the name Pterostichus coracinus in Table 6, to be
consistent with previous reports. In actuality, the taxon is a mixture
of P. coracinus and P. stygicus; males can be differentiated by the shape
of the penis, but we have so far been unable to find distinguishing
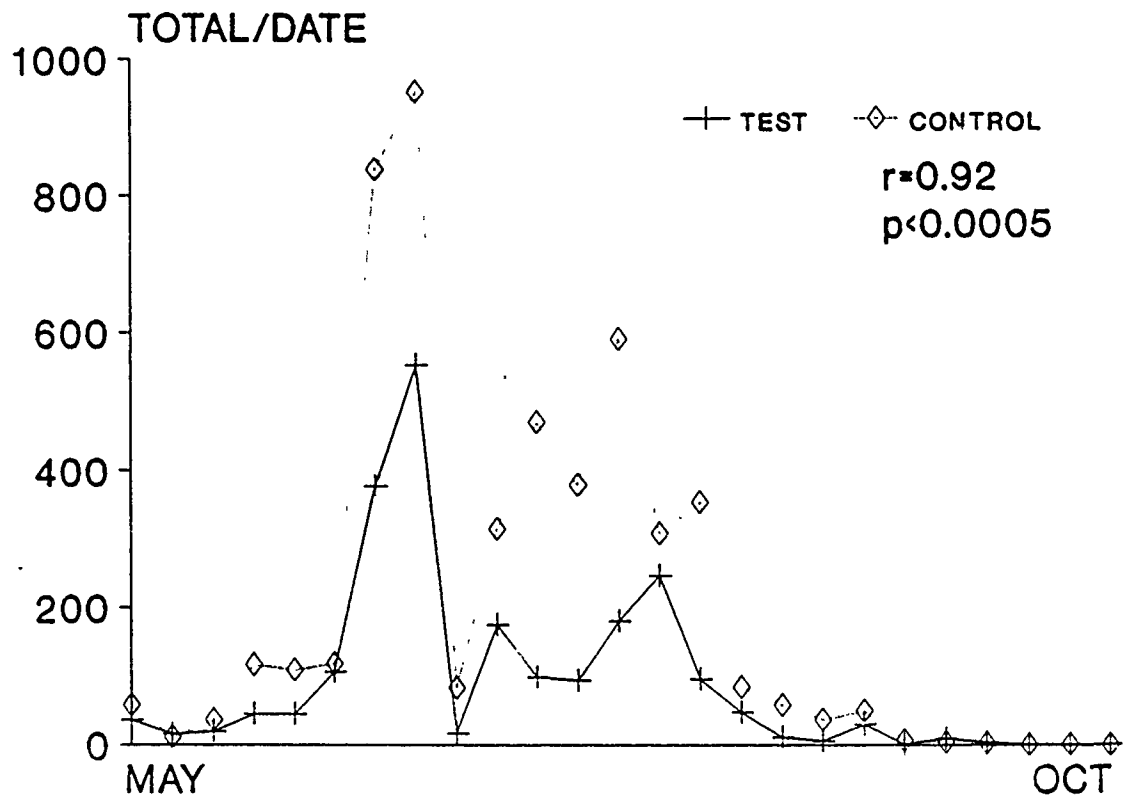
Fig. 15. Weekly trap catches of <u>Nanorchestes A</u> in Test and Control, 1988.

characteristics for females (confirmed by Gary Dunn, Dept. of Entomology, Michigan State University). The problem was first discovered during dissections for the purpose of egg counts; results were contradictory in terms of seasonal appearance of teneral and spent females.

Table 6. Total annual catches of Carabidae in Test and Control, 1985-88.

	TEST				CONTROL			
	1985	1986	1987	1988	1985	1986	1987	1988
P. melanarius	1087	1163	643	1528	183	222	356	299
P. coracinus	146	163	134	61	263	450	335	306
P. pensylvanicus	206	179	102	74	278	247	176	130
P. adstrictus	19	6	11	3	253	172	106	18
P. adoxus	2	14	20	4	30	20	22	9
P. mutus	232	203	210	102	24	15	26	12
Calathus spp.	81	40	32	23	290	157	130	71
C. frigidum	67	139	406	132	29	107	185	31
S. impunctatus	103	261	104	74	700	894	367	157
A. retractum	17	20	26	3	13	3	-	-
A. decentis	17	23	17	6	73	58	37	15
H. fuliginosus	76	139	71	124	55	116	61	88
C. fossor	49	49	52	48	5	6	7	3
C. cribricollis	25	54	23	24	35	115	66	44
N. aeneus	31	34	44	41	46	26	36	3
M. cyanescens	1	4	3	3	14	14	6	3
S. lecontei	5	5	10	-	9	13	10	13
A. placidum	2	-	-	-	-	-	-	-
T. quadristriatus	1	4	1	1	-	1	-	-
C. sylvosus	1	-	-	-	3	3	11	8
B. quadrimaculatum	-	-	3	2	2	-	1	-
H. fulvilabris	-	6	1	4	2	-	-	10
M. americanus	-	-	-	2	-	-	-	-
H. lewisi	-	-	-	1	-	-	-	2
A. permundus	-	-	-	1	-	-	-	-
Dyschirius sp.	-	-	-	2	-	-	-	-
Amara sp.	-	-	-	8	-	-	-	1
TOTAL ALL SPP.	2168	2506	1913	2261	2307	2639	1936	1222
TOTAL N SPP.	21	20	20	24	20	20	18	20

3.2. Diurnality

Of the species commonly captured in either site, Some are mainly or strictly nocturnal (_Cymindis cribricollis_, _Pterostichus mutus_), while _Notiophilus aeneus_, _Clivina fossor_ and _Harpalus fuliginosus_ have shown consistent preference for daytime activity (Table 7).

In 1988, deviations from previous years' patterns were noticed: _Pterostichus pensylvanicus_ and _Calosoma frigidum_, to a lesser degree also _P. mutus_, showed less diurnal activity than in previous years (Table 7). All three are strictly spring-breeding, and became increasingly nocturnal during two or three unusually warm nights in late May and early June (average air temperatures above 19°C). This evidence, although qualitative, allows us to explain year-to-year variation in diurnality of species which are flexible in their activity patterns.

Table 7. Percent diurnality (day catch / total catch x 100)of species commonly captured in either site, 1985-1988.

	TEST				CONTROL			
	85	86	87	88	85	86	87	88
P. melanarius	71.8	47.7	34.7	38.6	73.8	50.0	31.2	39.1
P. pensylvanicus	33.5	26.8	31.4	16.2	36.0	33.6	32.4	18.5
S. impunctatus	50.5	37.9	22.1	36.5	36.4	31.9	34.1	33.1
C. frigidum	98.5	85.6	80.8	56.1	86.2	86.0	71.4	38.7
H. fuliginosus	86.8	82.7	81.7	82.3	89.1	87.1	85.2	82.9
N. aeneus	100.0	100.0	100.0	97.6	95.7	100.0	97.2	100.0
C. cribricollis	0.0	0.0	0.0	0.0	0.0	0.0	1.5	2.2
P. mutus	19.8	15.3	19.5	13.7	-	-	-	-
C. fossor	89.8	71.4	80.8	83.3	-	-	-	-

3.3. Seasonal activity patterns

Summed bi-weekly totals (sums of 2 trapping dates and of day + night

catches) of reasonably common species (Figs. 16-21) serve to reiterate earlier comments. As expected, species-specific patterns of seasonality have been constant over the years, allowing distinction between spring-breeders (e.g., Fig. 16), usually with teneral adults appearing in the fall, and summer breeders such as P. melanarius (Fig. 19). Correlations between Test and Control have been excellent, confirming not only the general synchronicity of activity patterns but also of their modulation by environmental factors.

3.4. Fecundity

Until the taxonomic problem of P. coracinus / P. stygicus is solved, we report only fecundity estimates for P. melanarius and P. pensylvanicus, 1985 - 1987. These dissections are not particularly time-consuming, but have been temporarily shelved to accomodate more pressing matters. Data for additional species and years should be available by summer.

In both sites, the proportion of P. melanarius females with fully developed ova peaks during 3-4 weeks in late July and early August. The annual mean number of eggs can vary slightly between years (Table 8); this should not surprise, since we have no control over replication, and the proportion of females sampled by traps at any given time is not constant. However, neither between years nor sites were the differences significant (P $>$0.05).

Similar results were obtained for P. pensylvanicus (Table 9), where numbers of eggs/ female averaged between 10 and 12, and differed neither between sites nor years. Females are active during a brief period of approximately 8 weeks. Particularly in May, their ovaries contain undeveloped eggs as well as fully ripe ova; after mid-June, ovaries with ripe eggs, but no more undeveloped ova, are observed more and more frequently.
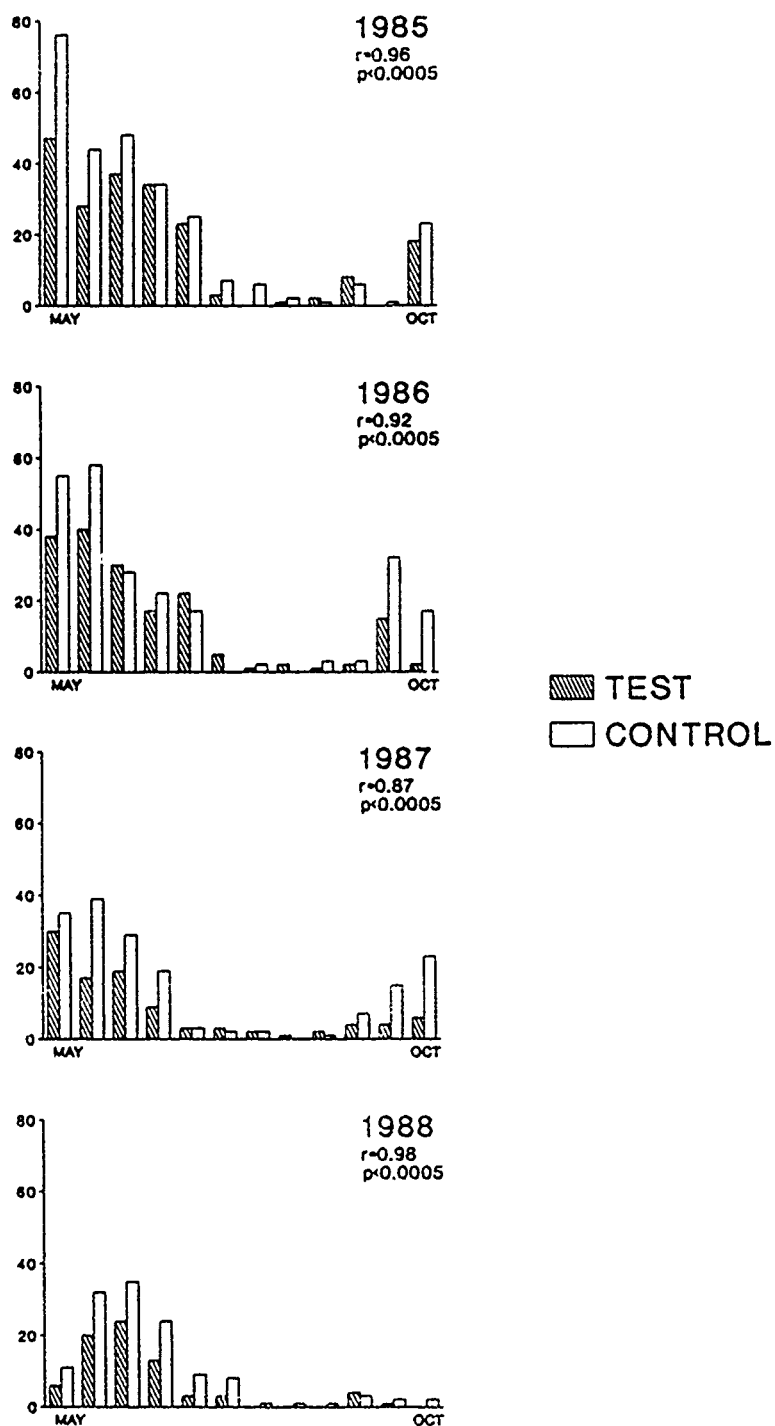
Fig. 16. Summed bi-weekly catches of _Pterostichus pensylvanicus_ in Test and Control, 1985-88.
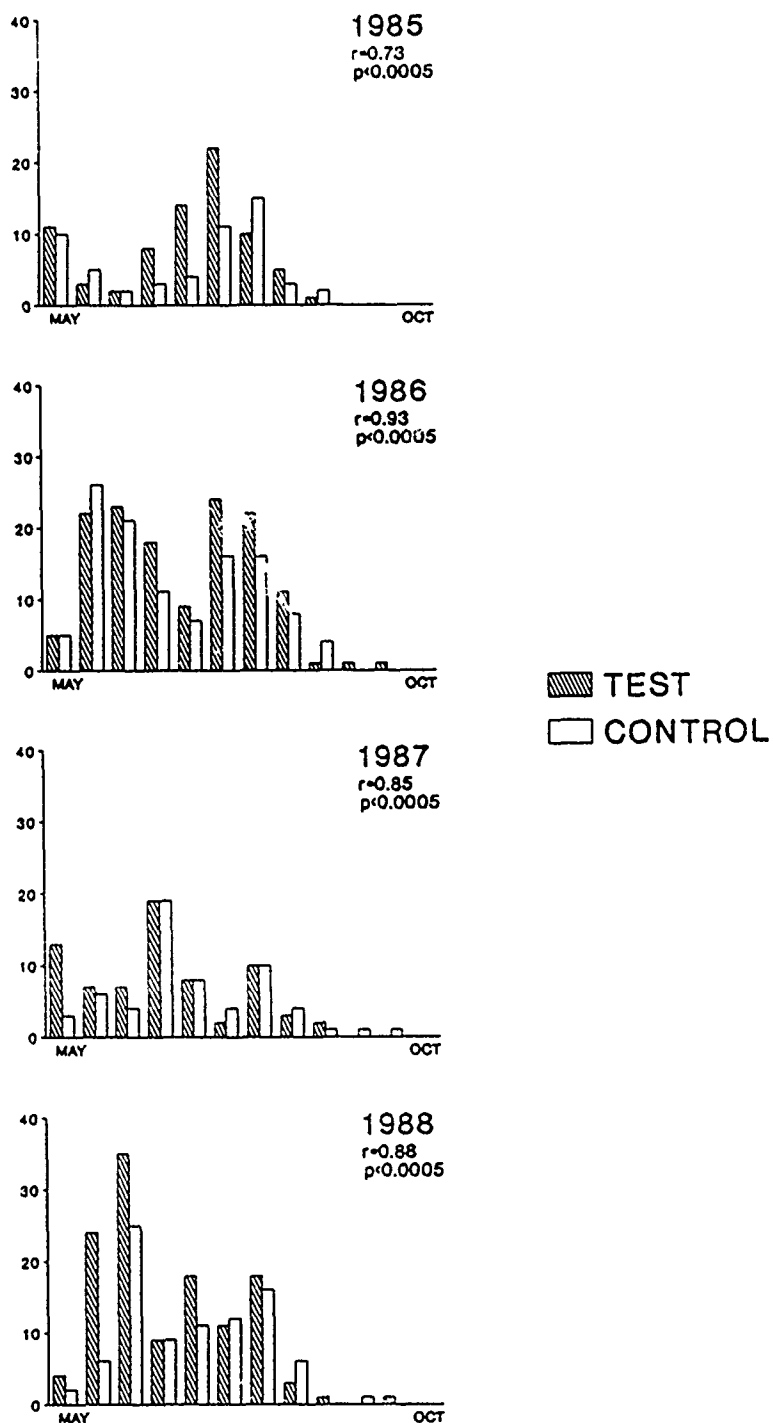
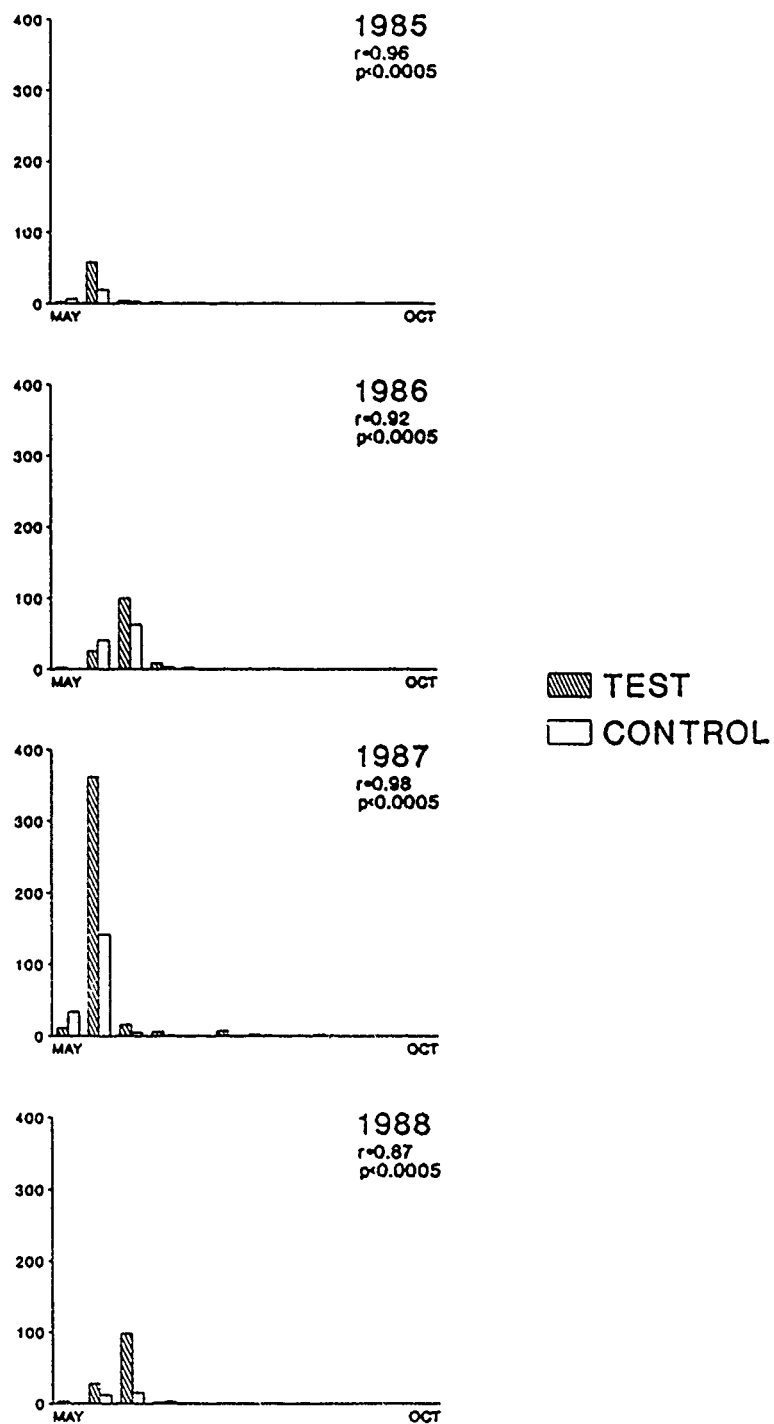Fig. 17. Summed bi-weekly catches of Harpalus fuliginosus in Test and Control, 1985-88.

Fig. 18. Summed bi-weekly catches of <u>Calosoma</u> <u>frigidum</u> in Test and Control, 1985-88.
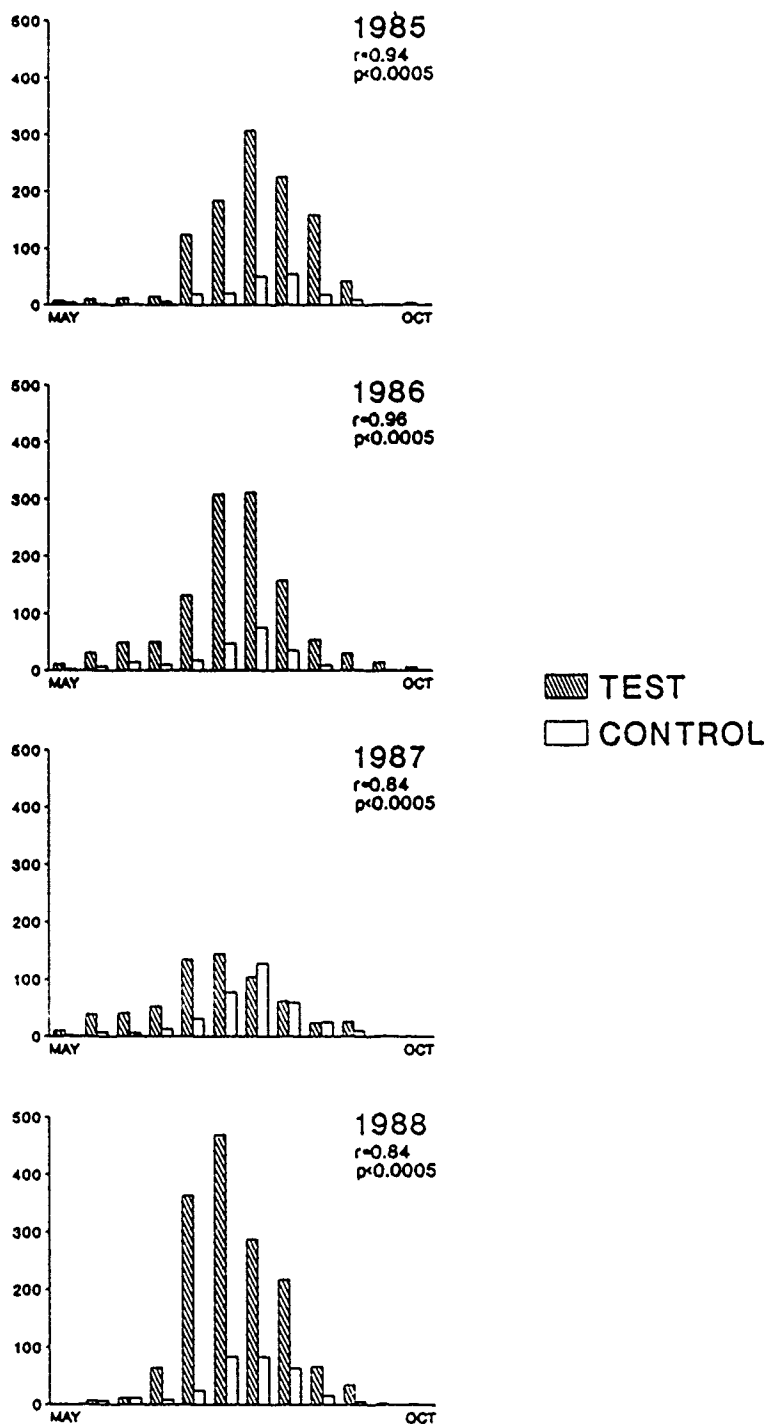
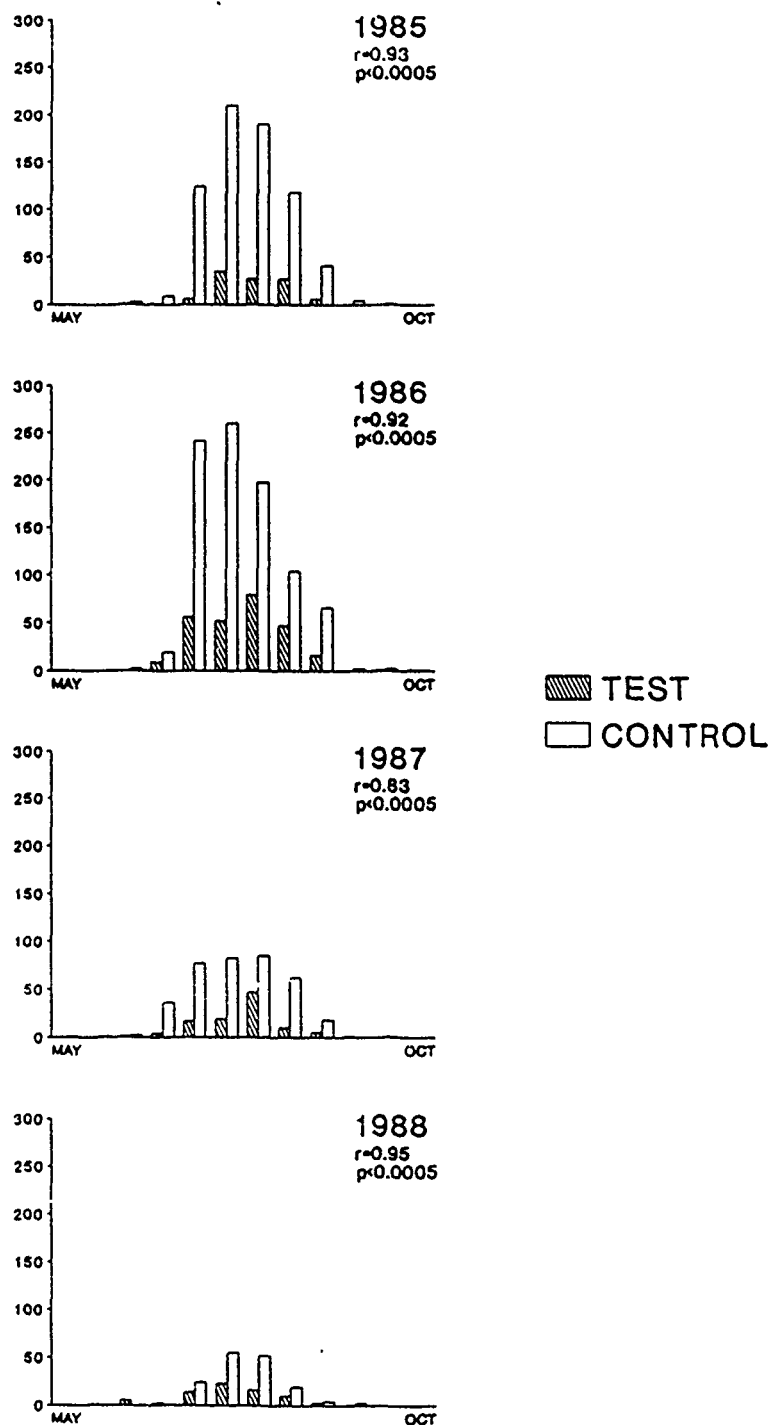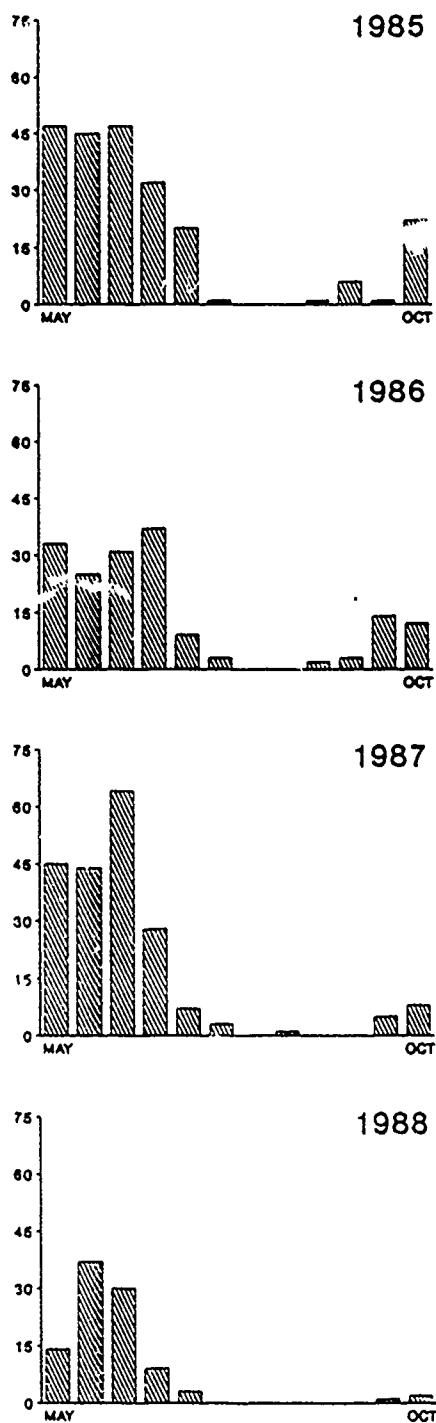Fig. 19. Summed bi-weekly catches of Pterostichus melanarius in Test and Control, 1985-88.

Fig. 20. Summed bi-weekly catches of <u>Synuchus</u> <u>impunctatus</u> in Test and Control, 1985-88.

Fig. 21. Summed bi-weekly catches of _Pterostichus mutus_, 1985-88.
The species is common only in Test.

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Table 8. Mean (± SD) annual number of ripe ova carried by <u>Pterostichus</u> <u>melanarius</u> in 1985-1987; N females dissected in parentheses.

	1985	1986	1987
TEST	14.18 ± 10.91 (141)	13.47 ± 19.91 (219)	12.75 ± 9.05 (138)
CONTROL	14.77 ± 8.88 (30)	13.85 ± 10.91 (47)	11.51 ± 10.21 (71)

Table 9. Mean (± SD) annual number of ripe ova carried by <u>Pterostichus</u> <u>pensylvanicus</u> in 1985-1987; N females dissected in parentheses.

	1985	1986	1987
TEST	12.61 ± 4.48 (67)	11.59 ± 5.33 (59)	11.63 ± 3.84 (38)
CONTROL	11.66 ± 4.12 (93)	10.04 ± 4.57 (49)	10.96 ± 4.32 (52)

· IV. EARTHWORMS

Lumbricid samples from the 1989 season have all been processed.

Specimens from the 1988 season first raised doubts about the true composition of lumbricid communities in Test and Control. _Aporrectodea trapezoides_, believed absent in Test, was discovered among Test material. In Control samples, a few individuals of _A. tuberculata_ were present. In order to correct possible identification errors, all earthworms (back through 1983) were then re-examined. Results are given in the following.

1. Lumbricid communities reviewed

1.1. Species composition

In addition to the dominant _A. tuberculata_ and its congener _A. longa_, two others are now known to exist in the Test site: _A. turgida_ and _A. trapezoides_, the endogeic species pair which dominates the Control community. Furthermore, 1989 (and only 1989) samples yielded a few cocoons of _Lumbricus terrestris_, a first and unquantifiable evidence of the species' presence in Test.

Among Control specimens, a small number of _A. tuberculata_ were found upon re-examination. Thus we now recognize that (with the exception of _A. longa_, still unique to Test) all other species are shared between sites, only in very different proportions. Omitting precise values, Table 10 serves to update our knowledge of lumbricid species composition.

Table 10. Species composition of lumbricid communities in Test and Control:

relative abundance ranging from "rare" to "dominant" (+++); - = absent.

	TEST	CONTROL
Dendrobaena octaedra	++	+++
Dendrodrilus rubidus	rare	rare
Lumbricus rubellus	++	rare *)
Lumbricus terrestris	rare	+ *)
Aporrectodea tuberculata	+++	rare
Aporrectodea turgida	rare	+++
Aporrectodea trapezoides	rare	++
Aporrectodea longa	++	-

*) Note: the two Lumbricus spp. are confounded
in Control, only adults and cocoons are dis-
tinguishable.

Prior to quantifying the development of these additional populations
during the project years, the following restrictions and potential
sources of error must be discussed:

a) In Test, the rare A. turgida can be distinguished from A. tuberculata
only if genital tumescences are present in the clitellar region. Thus
immatures of the two species will be confounded. Immatures of A. longa
and A. trapezoides also cannot be separated by external characteristics
(grey-brown pigmentation is present but variable in both); subadults and
adults are again distinguished by the location of genital tumescences
and, usually, by size.

b) In Control, the obverse is true: immatures of the dominant
A. turgida and the rare A. tuberculata are confounded, while adults
and subadults can be reliably identified.

c) With respect to cocoons, those of L. terrestris and L. rubellus are clearly different; those of Aporrectodea spp. are separated mainly by shape and weight, although some small degree of error is probably unavoidable (refer to section IV.3.).

In Table 11, the degree to which population estimates of the affected species pairs may be biased is presented in terms of the number of "adults" (aclitellates + clitellates + postclitellates) recovered per year.

Table 11. Total number of adult Aporrectodea spp. recovered from 1983-1989 samples in Test and Control; frequency of the rare species of each pair given as percent of total N.

	1983	1984	1985	1986	1987	1988	1989
CONTROL							
turgida	450	447	446	429	307	432	525
tuberculata	3	1	3	16	13	26	37
% of total	0.66	0.22	0.67	3.59	4.06	5.68	6.58
TEST							
tuberculata	255	547	689	637	552	678	585
turgida	0	0	2	1	0	5	4
% of total	0	0	0.29	0.16	0	0.73	0.68
TEST							
longa	30	45	73	72	43	79	69
trapezoides	3	8	11	17	10	23	29
% of total	9.09	15.09	13.10	19.10	18.87	22.55	29.59

In Test, abundance of A. longa cannot be reliably calculated, because immatures of A. trapezoides, judging by the high frequency of adults,

provide significant confusion. However, A. longa has been largely
ignored for the purposes of this project because its deep-burrowing
habits preclude accurate population estimates.

Numbers of A. turgida in Test were insignificant, so that estimates
of the very abundant A. tuberculata remain reliable.

In Control, up to 6.6% of unpigmented endogeic Aporrectodea consisted
of A. tuberculata. Unless the species increases drastically, the problem
is not yet serious and can be taken into account if necessary.

1.2. Population changes over time

Mean annual densities (1983 data based on four late-season sampling
dates) of the rare endogeics are given in Table 12.

Table 12. Mean annual abundance/ m^2 of the low-density endogeics dis-
covered in Test and Control (immatures not included).

	1983	1984	1985	1986	1987	1988	1989
tuberculata (C)	0.60	0.13	0.31	1.64	2.04	3.47	4.55
turgida (T)	0.0	0.0	0.20	0.10	0.0	0.67	0.49
trapezoides (T)	0.60	1.07	1.13	1.74	1.57	3.07	3.57

Aporrectodea turgida occurred only sporadically in Test; in 1988,
for instance, less than 8 in 1000 Aporrectodeas belonged in this species.

The A. trapezoides population, on the other hand, increased steadily
over the years, from a low of 0.6/ m^2 in 1983 to 3.6/ m^2 in 1989.
Virtually all individuals were postclitellates or large immatures with

faintly developed genital markings, which is probably the reason they were overlooked in the first place.

In Control, A. tuberculata also showed a steady numerical increase over the years, reaching 4.5/ m^2 in 1989.

If the trend of numerical increases of A. tuberculata in Control and of A. trapezoides in Test continues, then both communities are becoming more diverse, and will. come to resemble each other to a greater degree.

1.3. Spatial distribution of rare endogeics

In 1983, all 20 quadrats were sampled 4 times. In 1989, we switched from odd- to even-numbered quadrats in mid-season, so that all were again sampled at some time of the year. Intervening years' data are · of limited use, since samples stemmed from either odd- or even-numbered quadrats in a given year. While Table 12 presented mean densities for each site (based on the total N samples taken per year per site), Figs. 22 and 23 show quadrat-specific abundances (based on N samples taken in each quadrat per year).

Too few individuals of A. turgida were present in Test samples to determine any spatial particularities, although they stemmed mainly from quadrats at the upper edge of the site.

Starting in a narrowly defined area of Test, A. trapezoides has progressively spread through the site (Fig. 22). In Control, A. tuberculata was initially recovered from two opposing edges; by 1989, the species was found in a total of 11 quadrats. The epicenter of the population's advance was located in one corner, where the species also reached highest densities (Fig. 23).
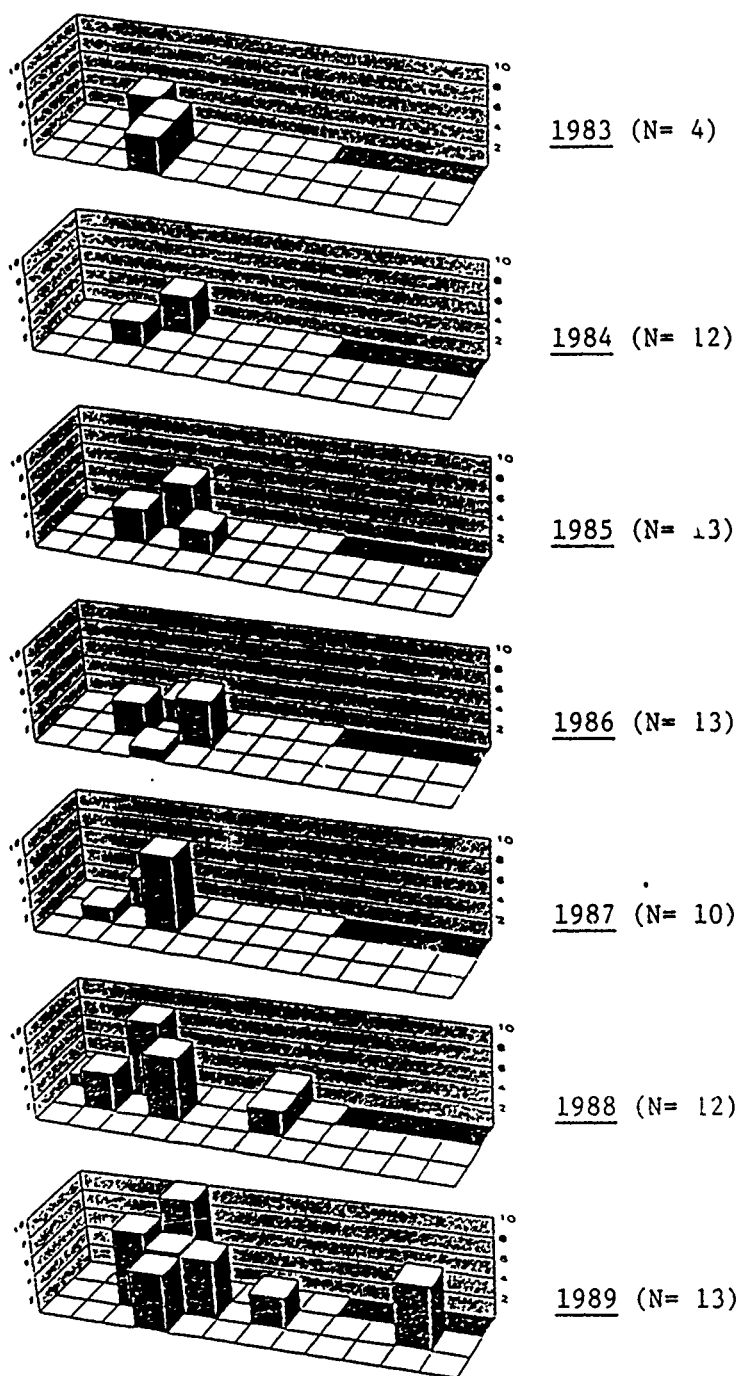
Fig. 22. Quadrat-specific density estimates for A. trapezoides in the

Test site, 1983-1989; number of samples taken /quadrat /year in parentheses.
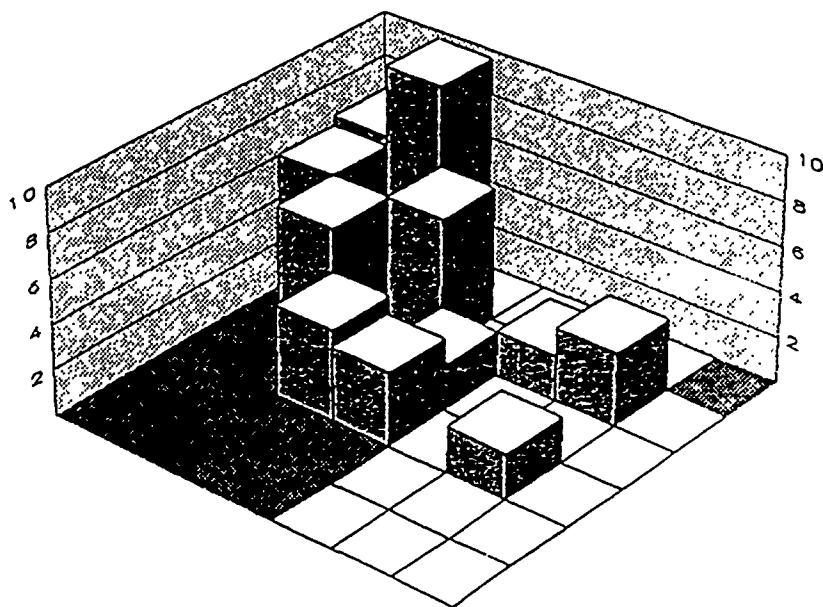
CONTROL 1983 *A. tuberculata*
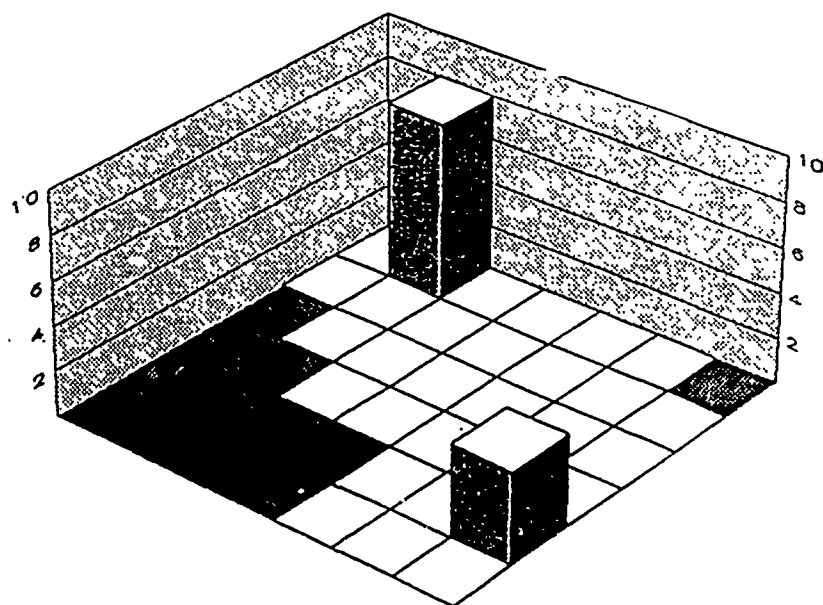
CONTROL 1989 *A. tuberculata*

Fig. 23. Quadrat-specific density estimates for A. tuberculata in the Control site: N = 4 samples/ quadrat in 1983; N = 13 samples in 1989.

We currently assume that these three species were always present in the study sites in very low numbers. Causes for the increasing abundance and spreading distribution of $\underline{A}$. trapezoides and $\underline{A}$. tuberculata are most likely connected to our activities in the sites; their exact nature, however, is not known.

2. Abundance of major species

Mean yearly density estimates for abundant lumbricids are presented in Fig. 24. Year-to-year fluctuations occur in all species, but are most pronounced in the epigeic $\underline{D}$. octaedra in Control. Potential underlying causes for numerical changes are discussed in subsequent sections. At this point we only make note of the slowly increasing abundance of $\underline{A}$. trapezoides in Control: are there effects of sampling activity at work, in analogy to the spreading and increase of the species in Test (Table 12, Fig. 23)?

3. Cocoon weights

Mean cocoon mass estimates (Table 13) reiterate information given in previous reports, adding 1989 data for comparison.

Mean mass of new cocoons in 1989 did not differ from previous years for any species, and neither did mass of $\underline{D}$. octaedra cocoons differ between Test and Control. It is noteworthy, however, that cocoon mass can vary between years in some respects. We compared 1987 through 1989 data in terms of frequency distributions over cocoon weight classes. Wide ranges in individual cocoon mass are characteristic of all species, the largest weighing approximately three times more than the smallest. Frequency distributions may shift within that range. Chi-square tests
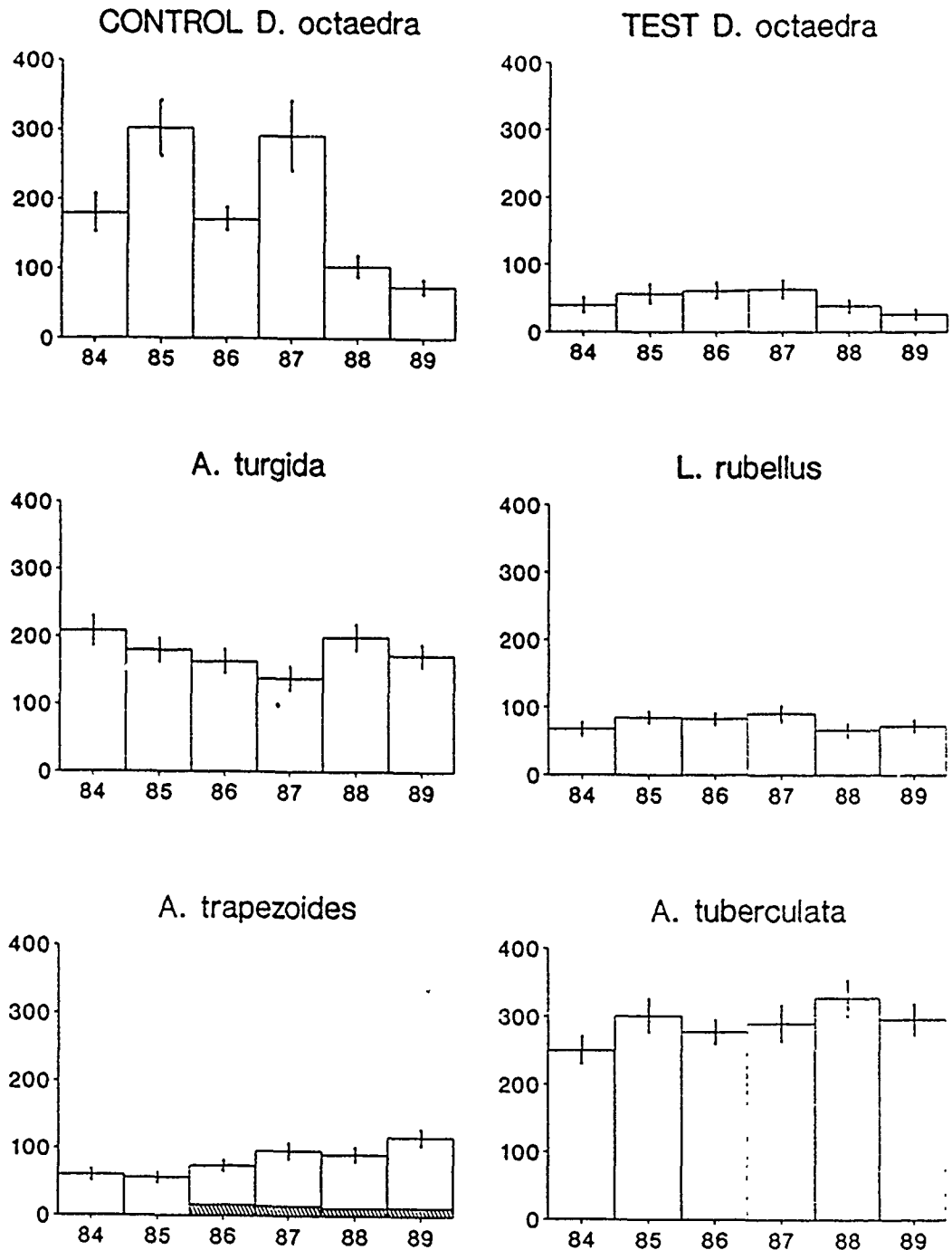
Fig. 24. Mean annual densities of abundant Test and Control lumbricids (± 95% CL); N samples = 120 in 1984 and 1988; N = 100 in 1987; N = 130 in 1985, 1986 and 1989. Shaded portion of bars, trapezoides: density in the 20-30 cm depth increment.

Table 13. Mass of new cocoons (mean ± SD) of Test (T) and Control (C) lumbricids; N cocoons in parentheses.

	1984	1985	1986	1987	1988	1989
octaedra T	3.48 ± 0.46 (307)	3.52 ± 0.50 (180)	3.44 ± 0.49 (241)	3.39 ± 0.49 (263)	3.41 ± 0.35 (101)	3.41 ± 0.48 (205)
octaedra C	3.55 ± 0.48 (772)	3.53 ± 0.48 (717)	3.48 ± 0.43 (673)	3.39 ± 0.44 (634)	3.32 ± 0.46 (516)	3.44 ± 0.52 (798)
rubellus T	9.40 ± 1.64 (322)	9.49 ± 1.62 (400)	9.43 ± 1.67 (460)	8.91 ± 1.64 (405)	9.09 ± 1.61 (232)	9.72 ± 1.80 (600)
tuberculata T	21.52 ± 4.08 (193)	19.59 ± 4.17 (137)	19.36 ± 4.94 (44)	20.73 ± 4.08 (223)	18.69 ± 4.01 (63)	20.99 ± 4.83 (181)
longa T	39.13 ± 4.51 (15)	47.86 ± 7.65 (14)	42.83 ± 8.19 (11)	42.73 ± 7.85 (13)	37.21 ± 6.28 (11)	40.13 ± 6.68 (40)
turgida C	11.79 ± 1.77 (174)	11.90 ± 1.93 (181)	12.07 ± 1.79 (129)	11.36 ± 2.08 (155)	11.40 ± 1.94 (102)	11.57 ± 1.85 (287)
trapezoides C	24.91 ± 3.44 (160)	24.79 ± 3.54 (125)	24.65 ± 3.23 (79)	24.43 ± 3.50 (186)	23.70 ± 4.13 (93)	25.22 ± 4.26 (509)

showed that distributions were not significantly different for L. rubel-

lus (Test) and A. trapezoides (Control), although a slight shift toward

higher weight classes was evident in both species (Fig. 25). Cocoons of

A. turgida and A. tuberculata (Fig. 26) exhibited the greatest year-to-

year variability, but only for A. tuberculata were these differences sig-

nificant. We suspect that cocoon weights tend to be lower in drier years

(1988), and that the upper tail of the distribution in 1989 may have con-

tained a few trapezoides cocoons (Fig. 26).

Dendrobaena octaedra, with relatively stable average cocoon mass

as well as invariant frequency distributions (Fig. 27) showed significant

differences neither between years nor sites.

4. Body mass of earthworms

In this section, we simply summarize the data on earthworm mass as they

will be used in subsequent discussions of population structure.

Preserved weights of hatchlings furnish the baseline from which further

developmental classes are derived. Fully developed cocoons of each species

(calices open, worm completely developed, no nutritive material present)

were dissected and the small juveniles were weighed. Their average weights

were doubled, to account for feeding after emergence, to arrive at hatchling

(class 0) weights (Table 14). Subsequent weight classes are generally

double the previous class, with the exception of D. octaedra, for which

the data indicated a smaller increment from class 0 to class 1. Data

in Table 14 are used in all following sections on frequencies or densities

of population segments.

L. rubellus   A. trapezoides

1987   1987

1988   1988
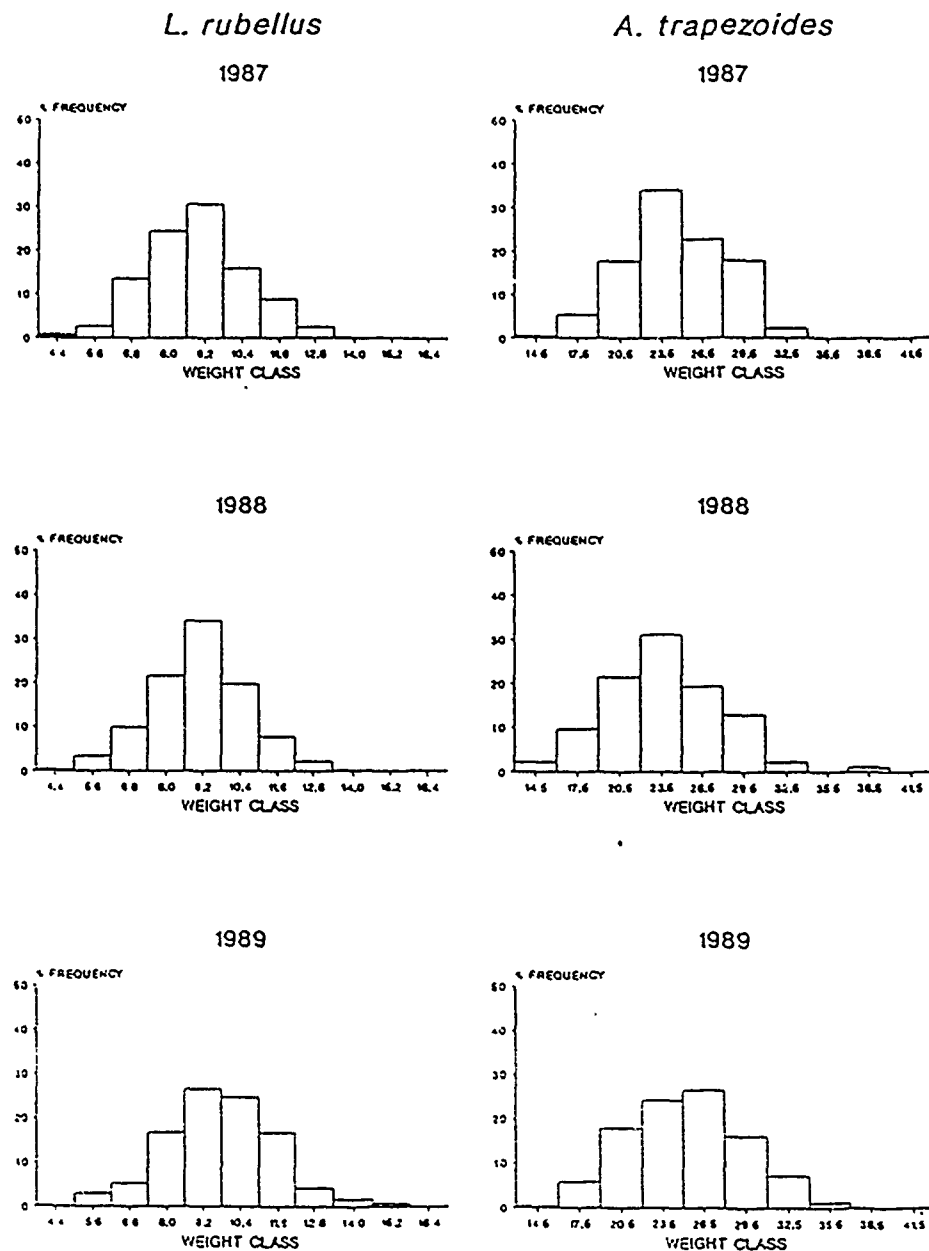

1989   1989

Fig. 25. Frequency distributions of cocoon weights of L. rubellus (Test) and A. trapezoides (Control); x-axis: weight class midpoints (mg).

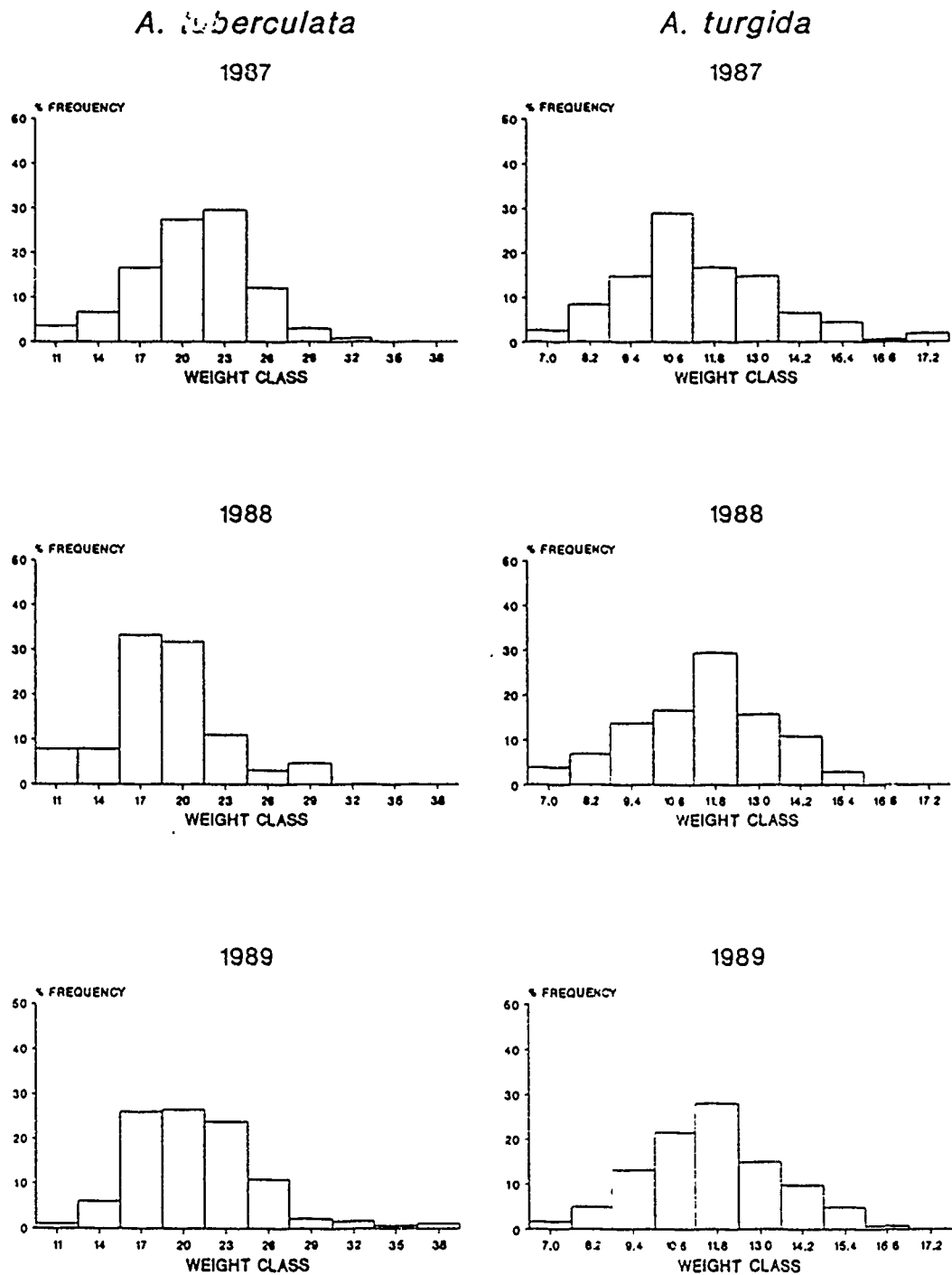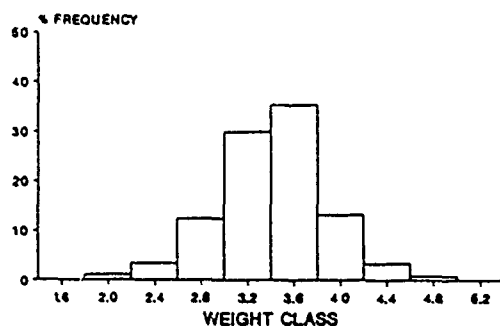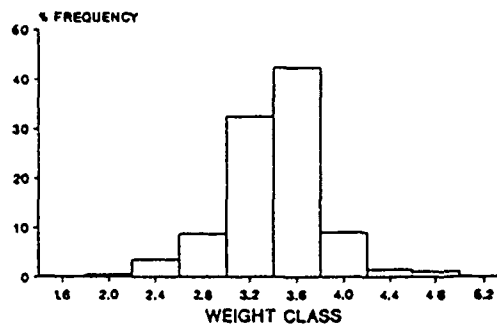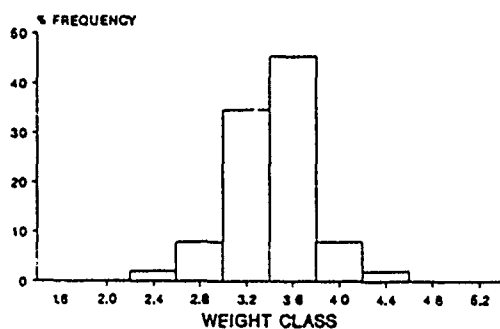## A. *tuberculata*

A. *turgida*

1987

1987

1988

1988

1989

1989

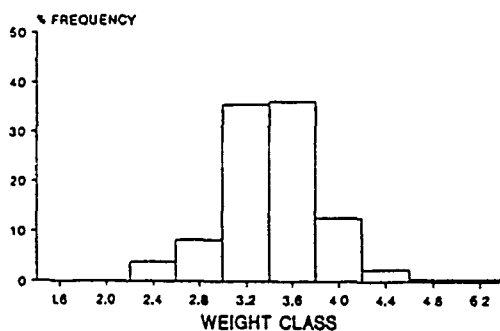Fig. 26. Frequency distributions of cocoon weights of A. tuberculata (Test) and A. turgida (Control); x-axis: weight class midpoints (mg).
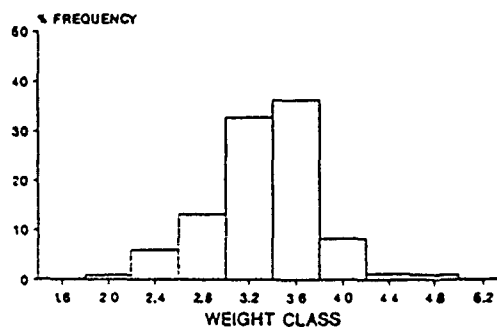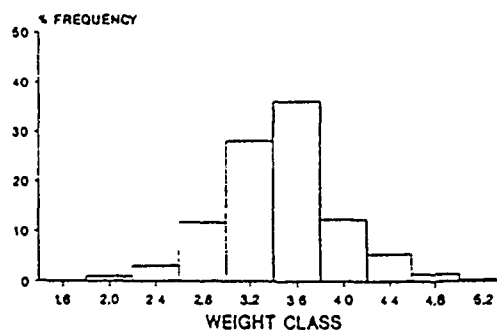
Fig. 27. Weight class frequencies (%) of cocoons of D. octaedra in Test and Control; x-axis: weight class midpoints (mg).

Table 14. Definition of weight classes (in mg) used for analyses of population structure; in parentheses: developmental stages falling into each class.

		OCTAEDRA	RUBELLUS	TUBERCULATA	TURGIDA
CLASS	0	8.0 (hatchling)	13.0 (hatchling)	22.0 (hatchling)	14.0 (hatchling)
CLASS	1	8.01 - 12.0 (immature)	13.01 - 26.0 (immature)	22.01 - 45.0 (immature)	14.01 - 28.0 (immature)
CLASS	2	12.01 - 24.0 (immature)	26.01 - 52.0 (immature)	45.01 - 90.0 (immature)	28.01 - 56.0 (immature)
CLASS	3	24.01 - 48.0 (immature + some adults)	52.01 - 104.0 (immature)	90.01 - 180.0 (immature)	56.01 - 112.0 (immature)
CLASS	4	>48.0. (adults + some 'immatures)	104.01-208.0 (immature)	180.01-360.0 (immature)	112.01-224.0 (immatures + some adults)
CLASS	5		>208.0 (immatures + adults)	>360.0 (immatures + adults)	>224.0 (adults + some immatures)

5. Dendrobaena octaedra

5.1. Vertical distribution

In 1989, abundance of D. octaedra was much reduced in both sites. Although we quantified in earlier reports that small juveniles have the greatest tendency to invade leaf litter, we continue to use total populations for analysis of vertical distribution. Error introduced by frequently small numbers of animals is thereby reduced.

Vertical movement in response to litter moisture is relatively constant between years and sites (Table 15). A single outlying regression slope

was obtained for Control, 1985 (P <0.05). Anova of regression parameters showed that 1989 data did not differ from previous years in either site; neither did slopes and intercepts differ between sites in 1989.

Table 15. Regression parameters for vertical distribution of D. octaedra in Test and Control; x_i = ln (% litter moisture); y_i = log (p/1-p); p = proportion of total population present in litter on each data.

Year (site)	df	r	a	b	P
84 (Control)	10	0.86	-3.13	0.76	0.000
85	11	0.69	-1.88	0.40	0.009
86	11	0.95	-4.63	1.02	0.000
87	8	0.88	-4.01	1.00	0.001
88	10	0.81	-4.15	0.98	0.002
84-88 (C)	58	0.82	-3.60	0.84	0.000
89 (C)	11	0.62	-3.53	0.72	0.025
84 (Test)	10	0.87	-2.79	0.65	0.000
85	11	0.71	-2.04	0.50	0.007
86	11	0.88	-3.77	0.84	0.000
87	8	0.92	-2.95	0.70	0.000
88	10	0.77	-3.24	0.77	0.004
84-88 (T)	58	0.81	-2.96	0.69	0.000
89 (T)	11	0.65	-2.06	0.42	0.017

5.2. Reproduction and recruitment

Clitellate adults are sparse or absent in May and early June (Figs. 28 and 29). Their abundance during the remainder of the season is

dependent on moisture conditions as well as on the number of large immatures (aclitellates) available for advancement into the adult pool.

In most years, a large percentage of new and developing cocoons, which tend to accumulate in the fall, overwinter and complete development the following spring. "Old" cocoons containing fully formed juveniles are prominent only in May (Figs. 28 and 29). This stage is of very short duration, and old cocoon abundance cannot be directly related to hatchling abundance: if soil temperatures prior to the first sampling date are relatively high, emergence will already be under way.

Overwintered cocoons thus produce a first influx of recruits, which may continue through July and August, then declines in the fall (Figs. 28 and 29). Seasonal and yearly variation in hatchling abundance is pronounced. Not only does it depend on the reproductive rate of adults, but adverse moisture conditions affect newly emerged worms(which have the strongest tendency to colonize litter) directly and immediately. In addition, the more abundant $D.$ octaedra population in Control seems to be subject to intraspecific competition which can reduce survival and growth of small immatures.

Certain basic biological characteristics of $D.$ octaedra lend themselves to between-site comparison through correlation or regression analysis:

a) The yearly mean percent of adults in the clitellate state ($r = 0.87$): the parameter simply reflects the degree to which moisture conditions enhance or suppress reproductive activity; 1989 data were not outlying. Typically, however, the proportion of adults that become reproductive is slightly lower in Test than in Control (Fig. 30).

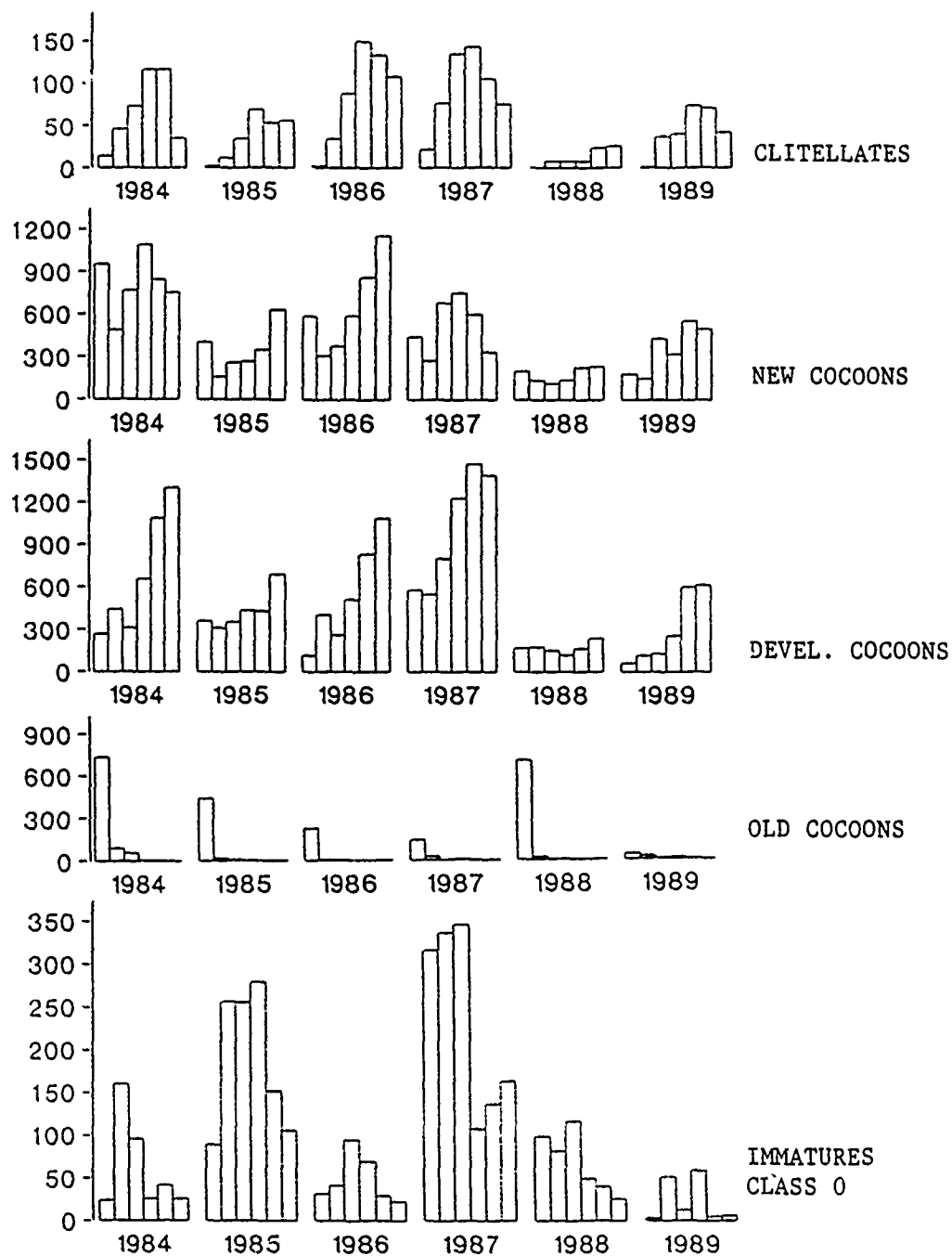b) The relationship between total number of clitellates (x_i) and total

Fig. 28. Dendrobaena octaedra, CONTROL: Mean seasonal (monthly) densities
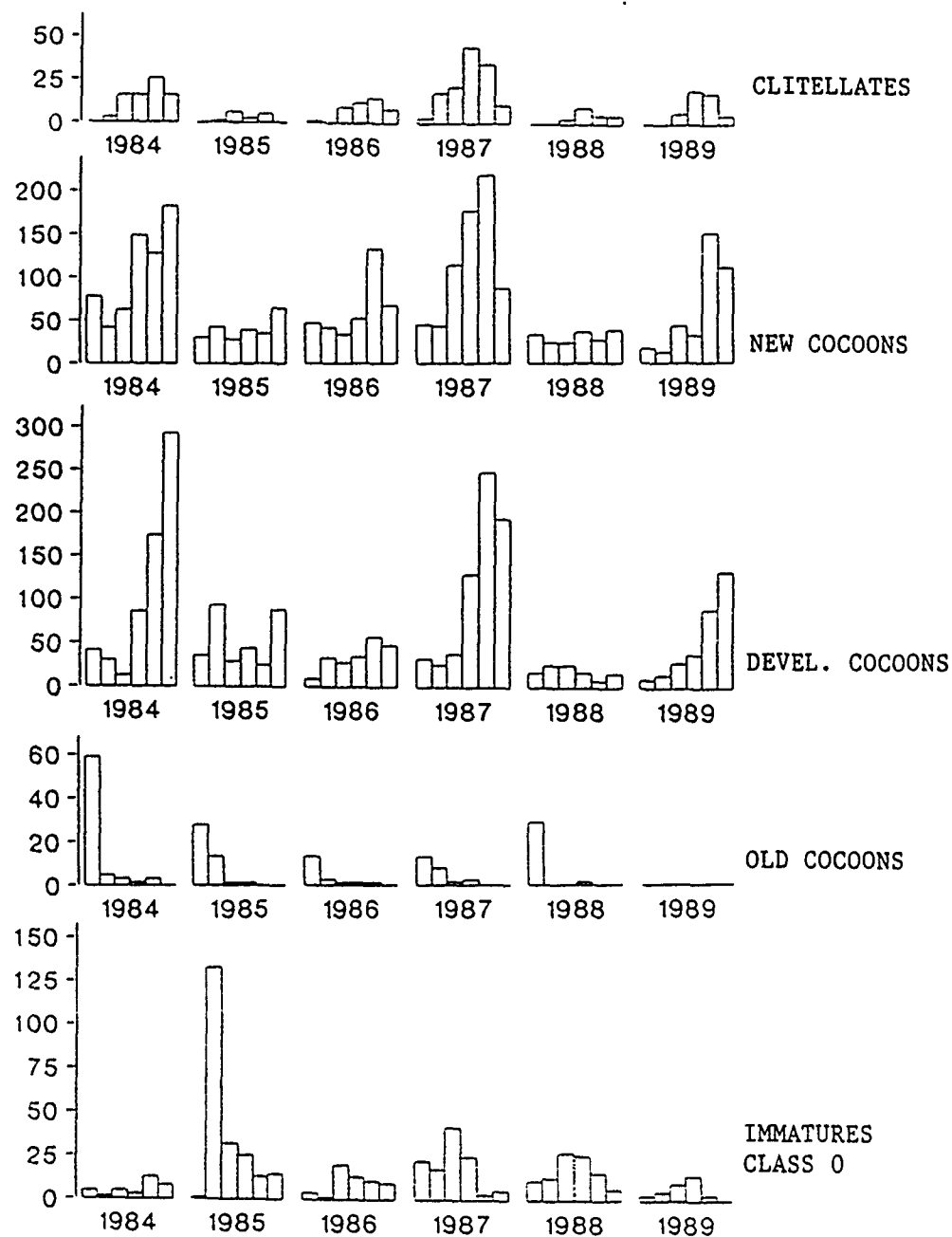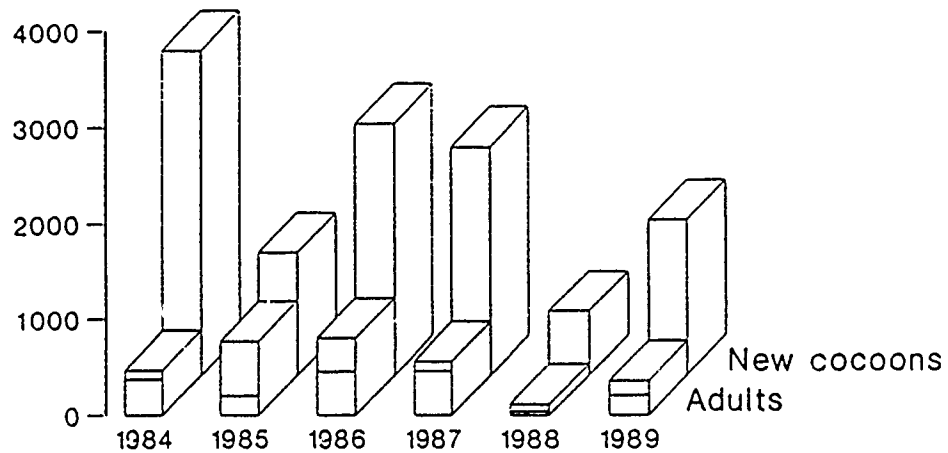of clitellates, cocoons and hatchlings, 1984-1989.

Fig. 29. <u>Dendrobaena</u> <u>octaedra</u>, TEST: Mean seasonal (monthly) densities of clitellates, cocoons and hatchlings, 1984-1989.
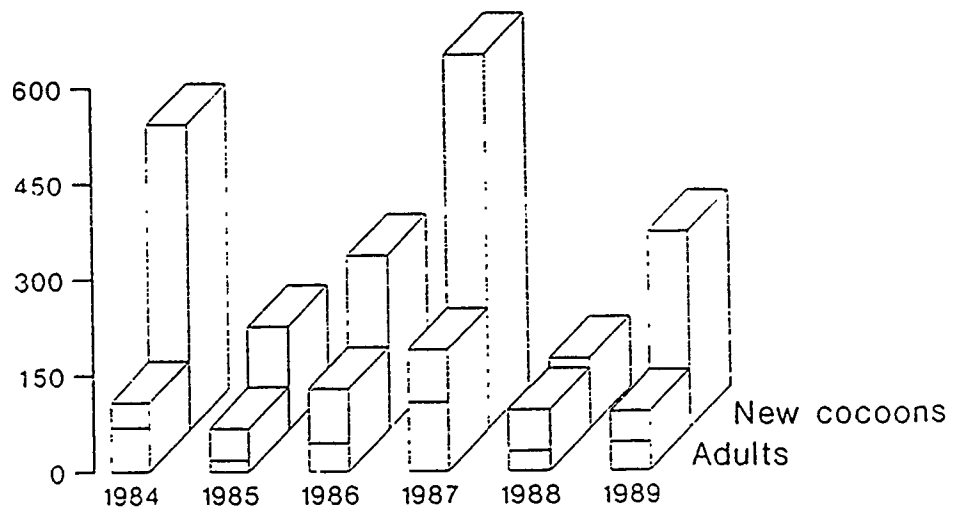
CONTROL *D. octaedra*

TEST *D. octaedra*

Fig. 30. Total number of adults (lower portion of bars = N clitellates) and of new cocoons of D. octaedra per year, 1984-1989.

number of new cocoons (y_i) (Fig. 30): only cocoon data from mid-June to late September are used, because spring and fall cocoon counts are over-estimates due to slow development and/or the presence of overwintered cocoons not related to the year's reproductive effort.

Test: $r = 0.97$, $y = 64.21 + 4.91 x$;

Control: $r = 0.86$, $y = 466 + 5.09 x$.

Regression slopes do not differ significantly, and 1989 data were well within previous years' limits.

c) The seasonal distribution (abundance) of developing cocoons, which results from a combination of: new cocoon inputs, which in turn are a result of reproductive rates in response to environmental factors; species-specific developmental rates as influenced by soil temperature; and hatching rates, which reduce their numbers.

Given the closely similar environmental conditions in Test and Control, year-specific fluctuations in densities of developing cocoons should be correlated between sites. With the exception of 1985 (reasons not yet explored) significant correlations were established between sites. Coefficients ranged from 0.78 (1986) to 0.96 (1987 and 1989), indicating that, irrespective of absolute densities, the seasonal developmental patterns of cocoons are a useful parameter for site comparison.

5.3. General patterns of growth and maturation

In this epigeic species, which has few means of escaping adverse conditions, tracking the development of a cohort to maturity is an almost impossible task (Figs. 31 and 32). By a combination of evidence, however, the following general pattern emerges:
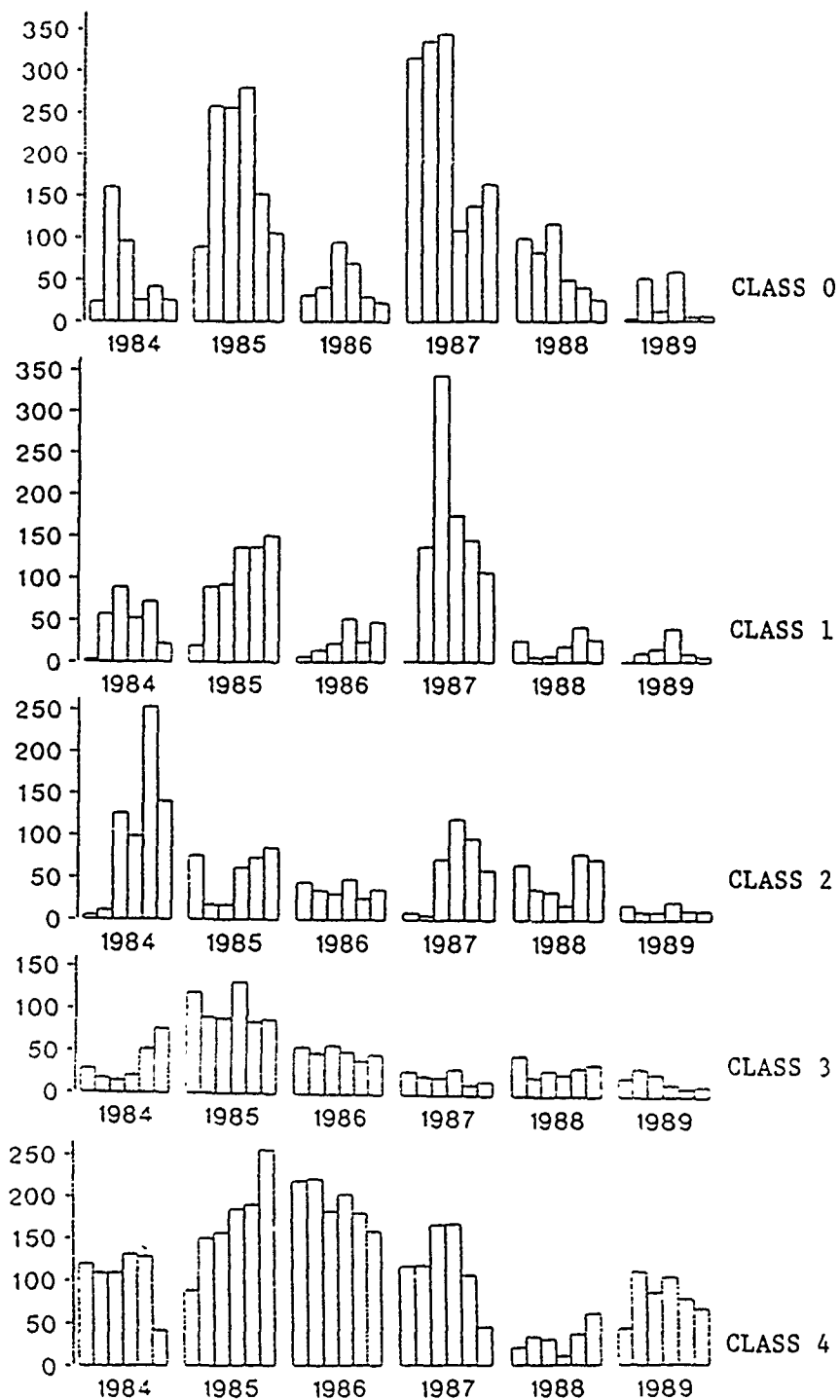
Fig. 31. <u>Dendrobaena</u> <u>octaedra</u>, CONTROL: Mean monthly densities of successive weight classes, 1984-1989 (see Table 14 for details).
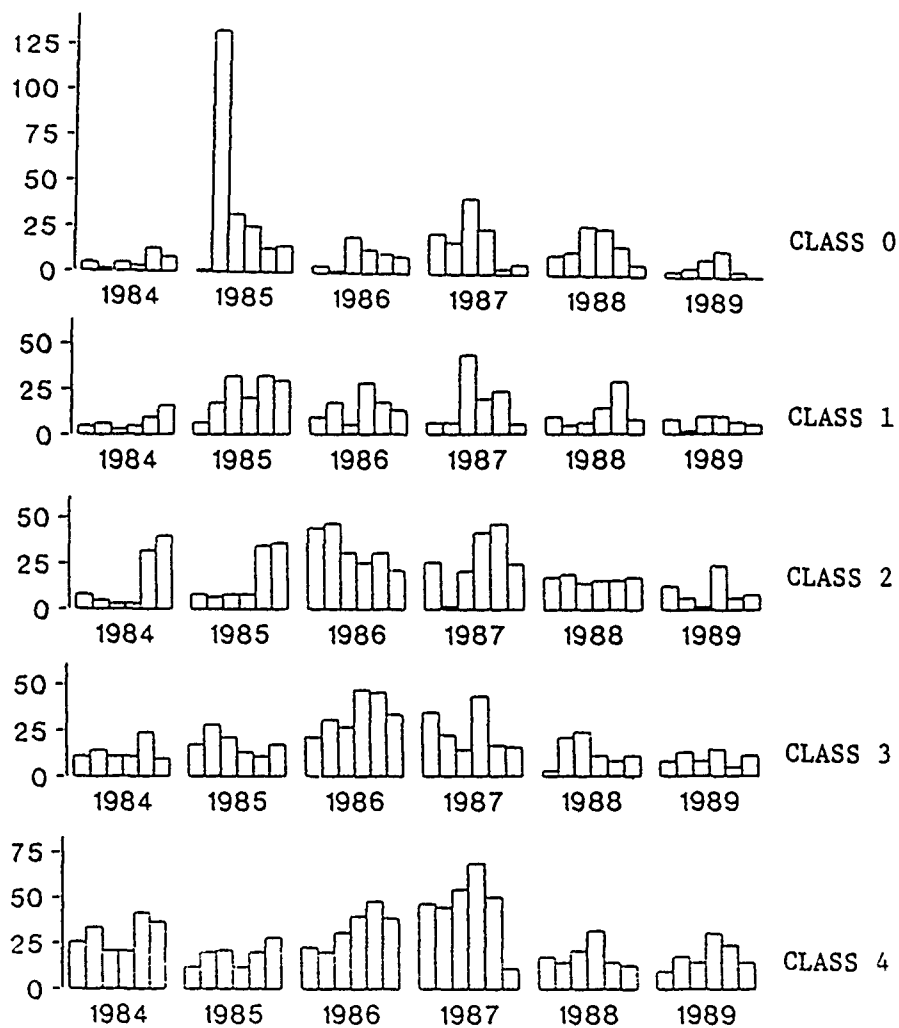
Fig. 32. <u>Dendrobaena</u> <u>octaedra</u>, TEST: Mean monthly densities of successive weight classes, 1984-1989 (see Table 14 for details).

- small juveniles stemming from the first half of the season (year 1) reach class 2 in July and August;

- in September and October, class 3 and, to a lesser degree, class 4 are attained;

- both classes 3 and 4 span the winter months, and represent large immatures and aclitellates at the beginning of year 2;

- adults present in year 2 stem mainly from the early-season cohort of year 1; these adults reproduce, if moisture conditions are propitious, and most will die at the end of year 2;

individuals which do not reproduce may survive a second winter and contribute to the adult contingent of year 3.

Circumstantial evidence for the postulated death of adults after they have reproduced is derived from seasonal body mass data (Fig. 33). There is a significant, gradual decline in mean mass of clitellates as the year progresses. We assume that these "spent" individuals will not survive the winter, and that each year's clitellate contingent essentially has to be replaced by the large immatures of the previous year.

5.4. Effects of rainfall patterns

We have been unable to establish statistical relationships between precipitation and population fluctuations. Seasonal timing, duration and severity of droughts all interact to produce varying effects on D. octaedra. However, two generalizations, and examples for them, can be derived from seasonal (Figs. 28,29,31 and 32) and annual summaries (Figs. 30 and 34) of population structure.
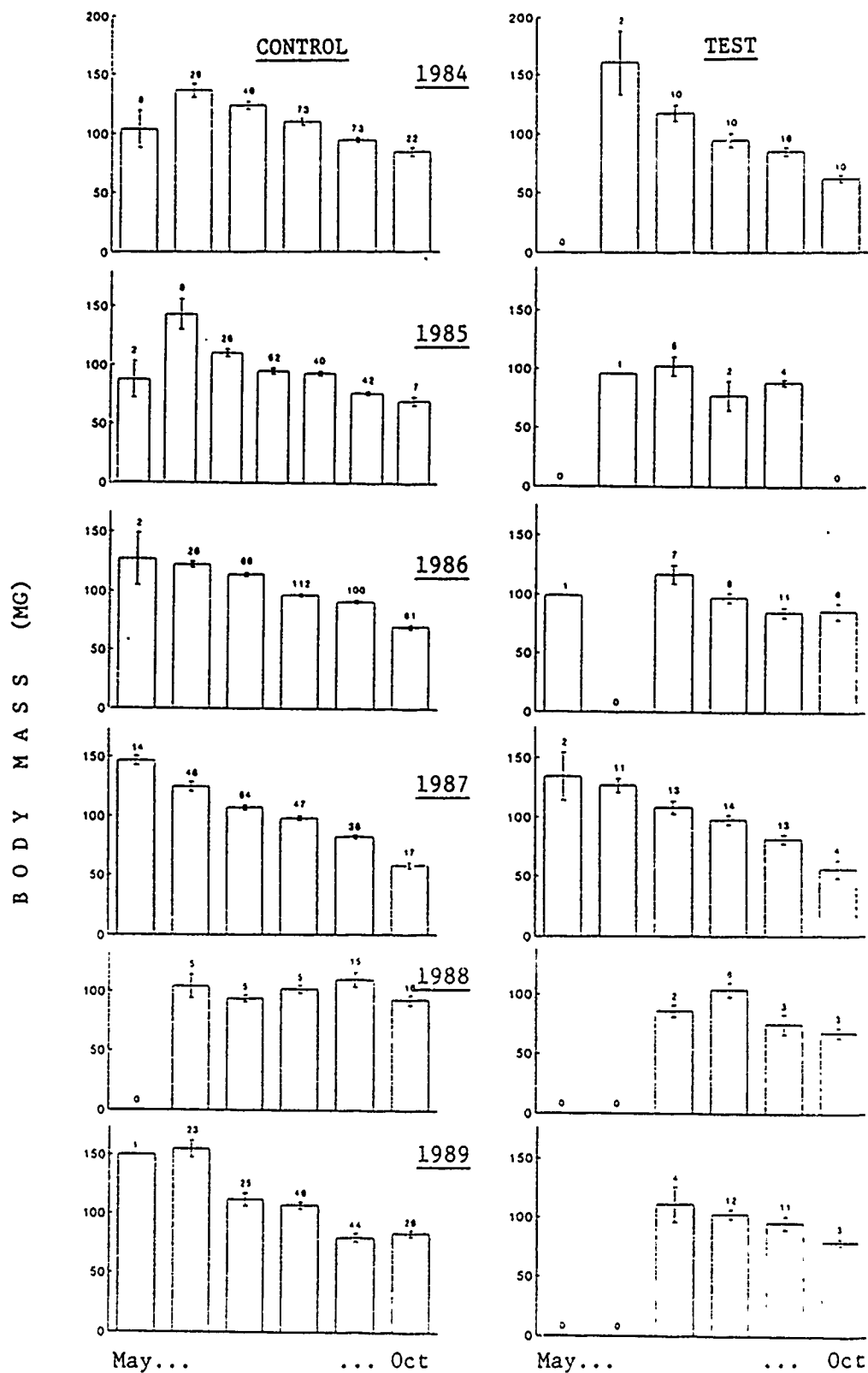
Fig. 33. Average weight of clitellate D. octaedra, specimens from every two sampling dates combined (May to October), 1984-1989. Number above each bar = N specimens.
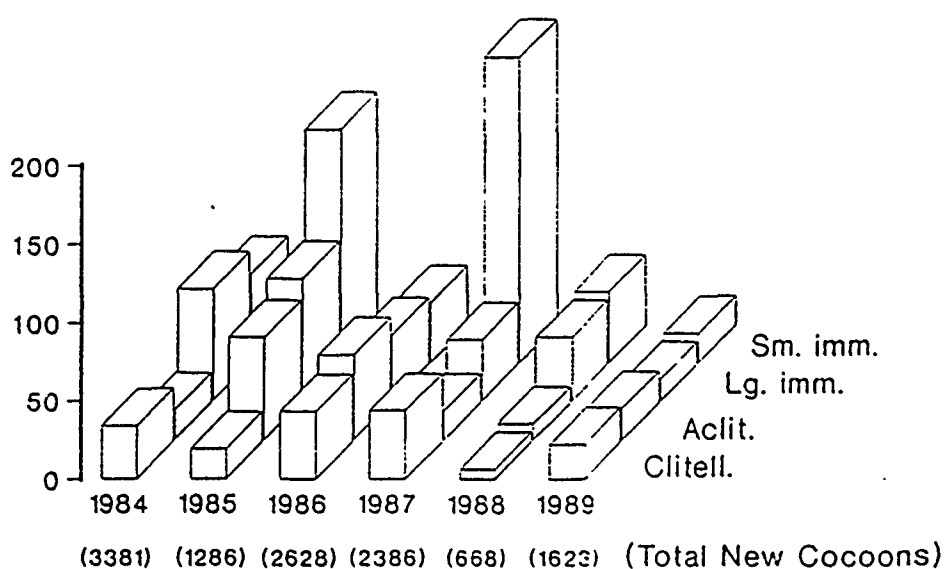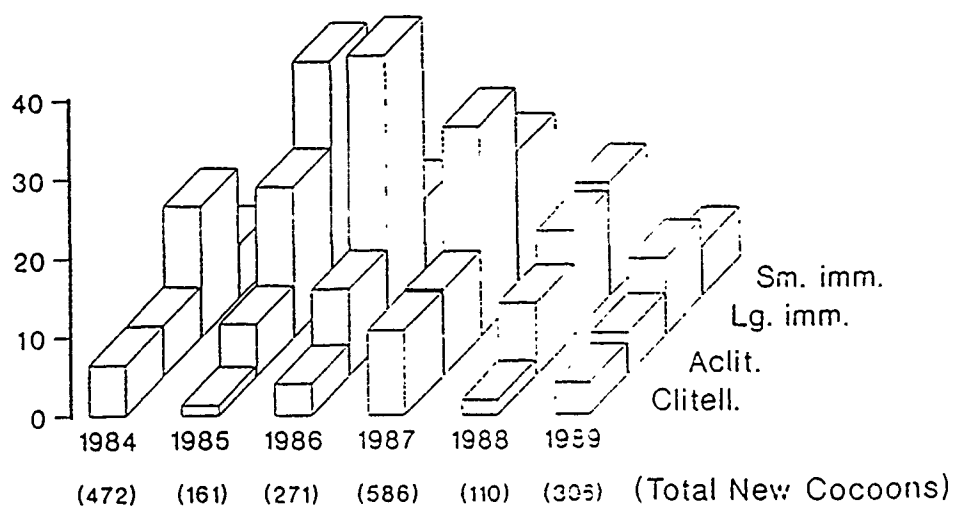
CONTROL *D. octaedra*

TEST D. *octaedra*

Fig. 34. Mean annual abundance of developmental stages of <u>D. octaedra</u> in Test and Control, 1984-1989: small immatures = classes 0+1, large immatures, aclitellates and clitellates defined by weight and sexual development.

a) Rainfall deficiencies in May and June, which were particularly severe in 1984, 1986 and 1988, affect mainly the survival of hatchlings and small immatures. A sizeable proportion of cocoons hatch in May and June; total cocoon production (included in Fig. 34 as a general reference) has a minor influence on small immature density when compared to spring drought effects.

b) Rainfall deficiencies which persist through July (1985, 1988) affect the proportion of adults which become clitellate and thus the production of cocoons. The effect can be alleviated (e.g., 1986, Fig. 30) if high adult abundance coupled with moderate fall temperatures allow a September-October reproductive peak.

In 1988, low precipitation resulted in unusually low litter and A horizon moisture for an unusually long time (late May through the beginning of August). Not only immatures, but adults as well appear to have suffered increased mortality at that time (Figs. 31 and 32), contributing to population declines in both sites.

5.5. Test vs. Control populations

It is obvious that population structure of D. octaedra differs between sites, despite strong similarities in reproductive parameters. Monthly density estimates of selected developmental classes (Figs. 31 and 32) show only occasional between-site similarities, and are statistically not useful; neither are yearly means (Fig. 34). However, it is necessary to develop an understanding of D. octaedra population dynamics since they are the sum of all population attributes.

Major differences in long-term trends:

a) As previously shown (Fig. 24), abundance of D. octaedra tends to fluctuate widely in Control; numbers are relatively stable in Test. Both populations declined in 1988 and 1989 due to drought-induced mortality and curtailed reproduction in 1988.

b) The proportionality of small : large immatures is particularly discrepant between sites: in Test, large immatures frequently outnumber small ones, in Control the opposite is observed (Fig. 34).

c) Adults tend to become clitellate later in the season in Test (e.g., Fig. 33).Although the very low numbers encountered in Test may mask the true onset of reproductive activity, the mean annual proportion of clitellates (of all adults) is consistently lower in Test. Densities of aclitellates were also less variable in Test (Fig. 34).

Based on the above evidence, we attribute the differences between Test and Control populations to the following mechanisms:

a) The Control population tends toward the limit of its potential maximum. In years with high densities of small immatures, intraspecific competition operates to reduce their numbers. In Test, intraspecific competition is much less likely, although competition with L. rubellus (also litter-feeding) may be one of the factors limiting expansion of D. octaedra in that site.

b) Resource requirements are more fully met in Control (ref. section V) than in Test. As a result, growth rates are lower in Test. and a greater proportion of large immatures are available for replenishing the adult contingent in the second year after emergence. Conversely, faster growth rates in Control tend to limit individuals' life span to two years, with less carry-over into a third year. Catastrophic environmental events

affecting survival and reproduction can therefore initiate more pronounced oscillations than in Test.

c) As a corollary to b), because of limited resources in Test, a smaller proportion of aclitellates matures to the clitellate state. Consequently, more aclitellates (large immatures) survive to the second year after emergence (assuming that adults die after reproducing). The magnitude of year-to-year fluctuations in adult densities is thereby moderated.

6. Lumbricus rubellus

6.1. Vertical distribution

Adults of the species are rarely found in leaf litter. Immatures, particularly the smaller individuals, show moisture-dependent vertical movement between litter and A horizon. Regression of the proportion of immatures in litter on litter moisture has yielded significant results for pre-ELF years, with r ranging from 0.62 to 0.96, and P= 0.03 or better (Table 16). Anova showed that slopes and intercepts did not differ significantly between years. On the average (1984-88) r = 0.77 at $P < 0.001$.

Data from 1989 diverged from established patterns. No significant relationship between moisture and immature distribution seemed to exist ($P > 0.2$, Table 16). At least in part, these results may be attributable to unusual population structure in 1989: small immatures were proportionally not well represented in 1989, due to low cocoon production in the drought year of 1988.

Table 16. Regression parameters for moisture-dependent vertical distribution of immature L. rubellus; x_i = ln (%litter moisture); y_i = log (p/1-p); p= proportion of immatures present in litter.

Year	df	r	a	b	P
84	10	0.62	-2.57	0.44	0.030
85	11	0.66	-2.73	0.44	0.014
86	11	0.79	-3.82	0.71	0.001
87	8	0.96	-3.47	0.69	0.000
88	10	0.90	-4.33	0.90	0.000
84-88	58	0.77	-3.33	0.62	0.000
89	10	0.36	-1.85	0.22	0.246

6.2. Reproduction and recruitment

Compared to other species, L. rubellus is relatively impervious to adverse conditions. Clitellate adults are present throughout the year, and production of cocoons, although modulated by environmental conditions, is never completely interrupted (Fig. 35).

Based on yearly mean densities (Fig. 36) the ratio of cocoon : clitellate abundance ranged from 4.7 (1988) to 7.4 (1987); for 1989, a ratio of 5.8 was obtained, which seems to be well within the normal range for the species. In addition, correlation between these two parameters was significant (r = 0.87), and 1989 data were not outlying.

Using biweekly estimates of cocoon and clitellate density, the relationship between them was barely significant for any given year, and soil moisture as covariate did not improve results. Without doubt, seasonally
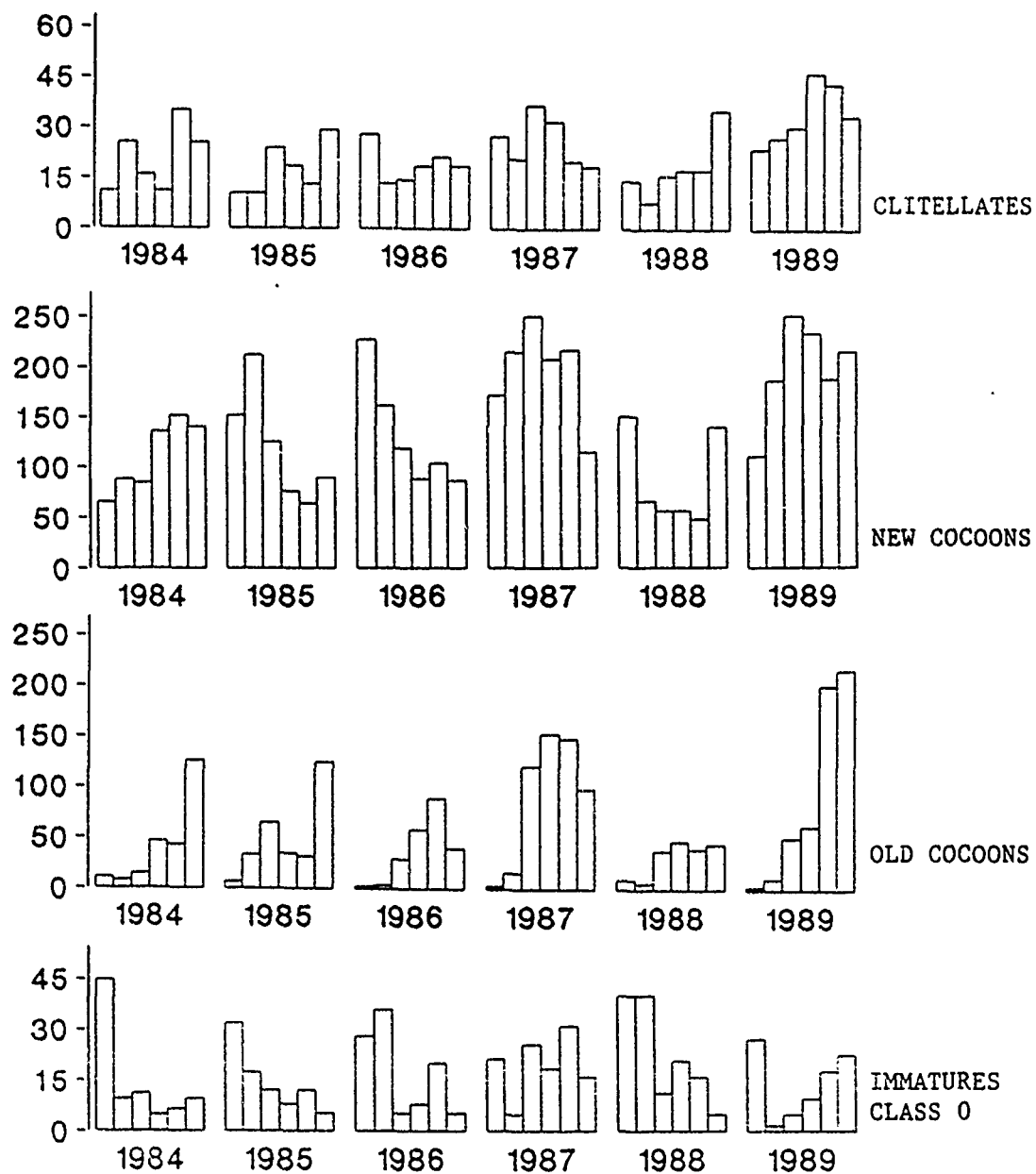
Fig. 35. Average monthly abundance of clitellates, cocoons and hatchlings of _Lumbricus rubellus_ in Test, 1984-1989.
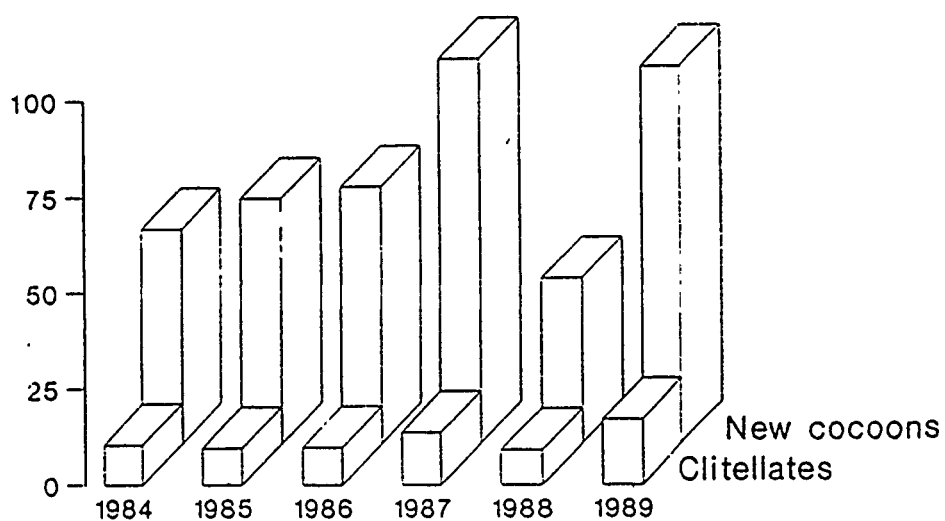
L. rubellus

Fig. 36. Mean annual density, m^2 of clitellate adults and cocoons of
L. rubellus in Test, 1984-1989.

variable rates of cocoon deposition provide one cause for lack of corre-
lation. In addition, we suspect that the clitellum in L. rubellus
persists longer than in other species, even if reproduction has slowed
or stopped.

Old cocoons containing fully developed worms are essentially absent
in the spring (Fig. 35), but begin to increase in July and August due to
cocoon inputs stemming from the spring of the same year. Old cocoons tend
to accumulate in October, and resulting peaks in hatchling abundance are
most pronounced in early May of the following year (Fig. 35). Evidently,
emergence of juveniles occurs prior to the first sampling date, at relati-
vely low soil temperatures.

The consequences of one year's weather patterns are thus most pronounced
in the spring of the following year. For example, high cocoon production
in 1987 resulted in large numbers of hatchlings in May and June of 1988.
Depressed reproduction in 1988 (soil moisture was below 20% for three
consecutive summer months) led to an isolated, relatively low emergence
peak in May of 1989 (Fig. 35).

6.3. Patterns of growth

Despite considerable variability, temporal patterns of development
can be discerned. In any year in which a major pulse of recruitment occurs
in May (e.g., 1984 or 1985), we observe class 1 immatures peaking in June
and July, class 2 in August and September, and class 3 in September and
October (Fig. 37). Juveniles may reach class 4 late in the same year
they hatched, or in the spring of the following year. Presumably, large
immatures (class 5) first become capable of reproducing during the fall
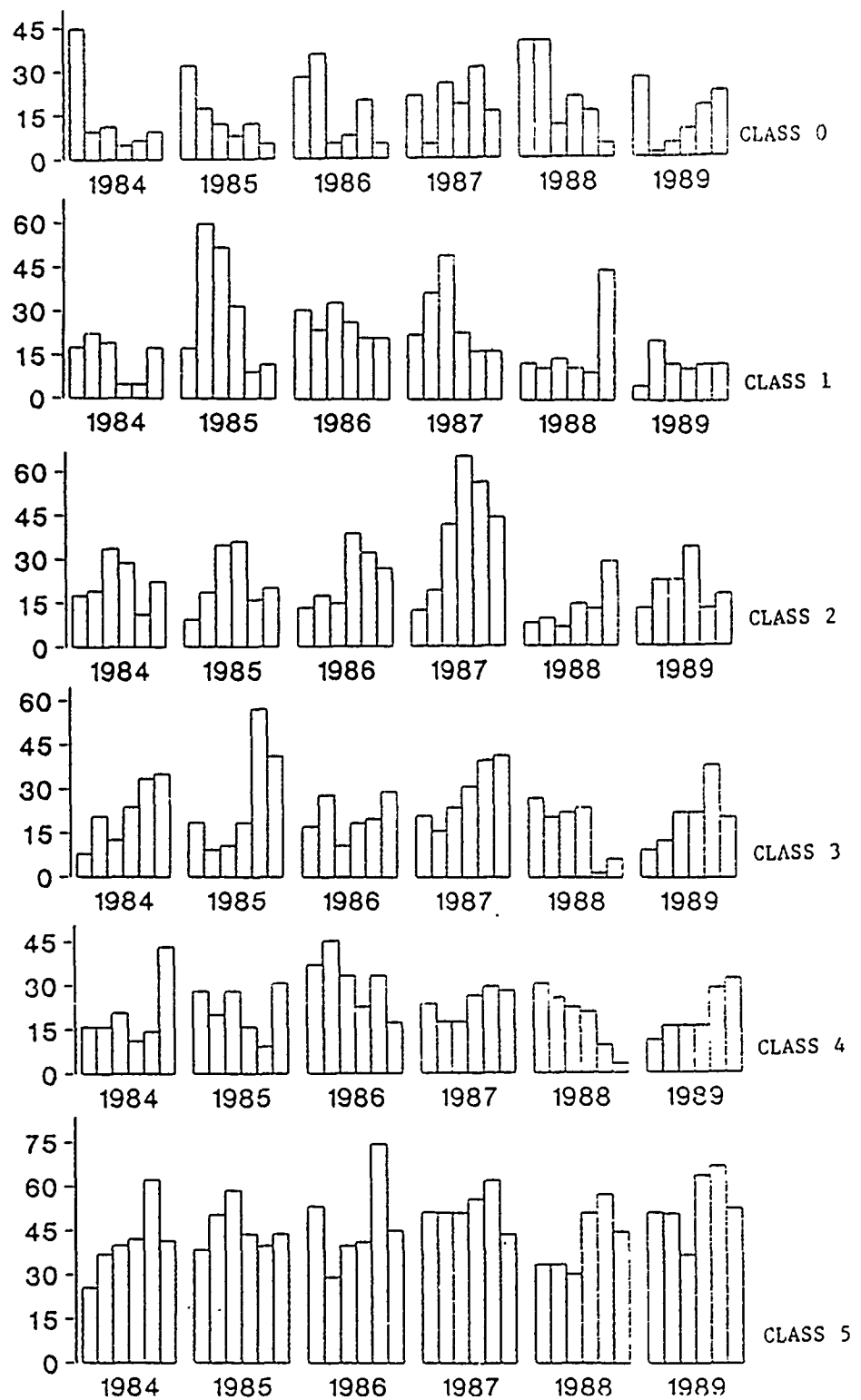of their second year, after approximately 1.5 years of growth. Consistently

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Fig. 37. Mean monthly abundance of L. rubellus by weight class (see
Table 14 for details).

high numbers of class 5 individuals indicate that they may survive, and reproduce, for 2 or 3 years after reaching adulthood.

Severe drought is reflected in a disruption of this pattern. High cocoon production in 1987 resulted in large numbers of recruits in May-June of 1988 (Fig. 35). Subnormal rainfall in 1988 then curtailed survival of that cohort, only small numbers of which were present in the fall of 1988 and spring of 1989 (classes 3 and 4, Fig. 37).

6.4. Annual population structure

Disregarding the seasonality of emergence and growth, one may follow the fate of a given cohort by cumulating seasonal density estimates over each year (Table 17). Interpretation of these data then leads to an explanation of overall mean annual density fluctuations (Fig. 24).

Table 17. Cumulative abundances of L. rubellus over 12 sampling dates per year (for weight class definitions see Table 14).

	1984	1985	1986	1987	1988	1989
New cocoons	667	719	785	1173	515	1179
CLASS 0	86	87	103	117	133	83
CLASS 1	86	183	157	165	104	75
CLASS 2	133	135	143	238	80	122
CLASS 3	134	156	124	174	102	126
CLASS 4	122	132	189	143	112	120
CLASS 5	249	276	284	316	251	323
Total worms	810	968	1000	1154	782	849

Overall population densities are determined by a combination of three main factors:

a) Abundance of new cocoons, which determines hatchling densities the following spring (as well as, to some degree, the same year they are produced); 1987-88 and 1988-89 furnish contrasting examples (Table 17).

b) Survival of small immatures during the year in which they hatched; e.g., compare population structure in 1987 with that in the drought year of 1988 (Table 17) in terms of the relative numbers in successive classes.

c) Survival of large immatures and longevity of adults; although speculative, our interpretation of the data indicates that the species is relatively long-lived. Class 5 individuals probably persist through the third and fourth year following their emergence, and provide a stabilizing factor for overall population abundance.

One may now cautiously predict that, given adequate rainfall, _L. rubellus_ will show a numerical increase in 1990, as a result of high cocoon production in 1989 (Fig. 35). Should 1990 be another dry year, the 1987-88 pattern of cohort suppression and concurrent low reproductive activity should be repeated.

7. Aporrectodea tuberculata and A. turgida

7.1. Vertical distribution

Comparing single-year data, we find the relationships between vertical distribution of _A. tuberculata_ and soil moisture to be most significant in the drier years (1985, 1986, 1988). For juveniles (Table 18) as well as adults (Table 19), neither 1984 nor 1989 data produced significant

regressions. Pending further observation in future operational years, we suggest that soil moisture at or above 20% is not a discriminant factor for endogeic distribution.

Table 18. Regression parameters for vertical distribution of juvenile A. tuberculata (proportion of total number in A horizon vs. % A horizon moisture).

Year	df	r	a	b	P
84	10	0.44	-0.23	0.03	0.149
85	11	0.80	-1.14	0.07	0.001
86	11	0.75	-1.66	0.10	0.003
87	8	0.71	-2.58	0.12	0.022
88	10	0.63	-0.53	0.05	0.029
84-88	58	0.60	-0.59	0.05	0.000
89	11	0.24	-0.44	0.04	0.442

Table 19. Regression parameters for vertical distribution of adult A. tuberculata dependent on A horizon moisture.

Year	df	r	a	b	P
84	10	0.37	-0.24	0.03	0.240
85	11	0.83	-2.88	0.12	0.000
86	11	0.88	-3.51	0.16	0.000
87	8	0.64	-3.06	0.13	0.045
88	10	0.85	-2.32	0.15	0.000
84-88	58	0.62	-1.70	0.09	0.000
89	11	0.19	-0.43	0.04	0.530

Juvenile and adult A. tuberculata show similar distribution patterns, neither regression slopes nor intercepts differing significantly (1984-88 data, P >0.15). Regression of the total population on soil moisture does not much increase r (r = 0.63, vs. 0.60 for juveniles and 0.62 for adults, 1984-88 data).

In 1989, the Control site received less rain than Test, and soil moistures were often lower and more variable than in Test (Fig. 2). For both juvenile (Table 20) and adult (Table 21) A. turgida, depth distribution was significantly related to A horizon moisture. Anova of regression parameters showed no between-year differences, and no differences between juveniles and adults.

As stated in earlier reports, A. tuberculata and A. turgida respond equally to moisture fluctuations (regression slope b = 0.06 for both species, 1984-1989 data).

Table 20. Regression parameters for vertical distribution of A. turgida immatures on soil moisture.

Year	df	r	a	b	P
84	10	0.87	-0.91	0.06	0.000
85	11	0.65	-0.61	0.04	0.016
86	11	0.78	-1.69	0.08	0.002
87	8	0.74	-1.63	0.09	0.014
88	10	0.64	-0.58	0.05	0.024
84-88	58	0.66	-0.74	0.05	0.000
89	11	0.80	-1.31	0.08	0.001

Table 21. Regression parameters for vertical distribution of A. turgida adults on soil moisture.

Year	df	r	a	b	P
84	10	0.70	−1.32	0.07	0.012
85	11	0.72	−2.04	0.09	0.005
86	11	0.60	−2.06	0.09	0.031
87	8	0.60	−1.58	0.09	0.068
88	10	0.70	−1.84	0.10	0.011
84–88	58	0.64	−1.61	0.08	0.000
89	11	0.78	−1.93	0.09	0.002

7.2. Reproduction and recruitment

Both of these endogeics are capable of uninterrupted reproduction all season long, but abundance of clitellate adults is strongly influenced by soil moisture.

Fluctuations in clitellate A. tuberculata densities tend to be more pronounced (Fig. 38) than those of A. turgida (Fig. 39).In both species, cocoon production may be fairly continuous (1987) or may peak in spring and/or fall depending on the timing and distribution of rainfall (e.g., 1984 vs. 1985, Figs. 38 and 39).

Cocoons produced in spring hatch in the fall of the same year, approximately 3 months later. Summer and fall cocoons overwinter and juveniles emerge the following year.

Fully developed cocoons and subsequent increases in hatchling abundance may thus occur at different times of the season (Figs. 38 and 39). In
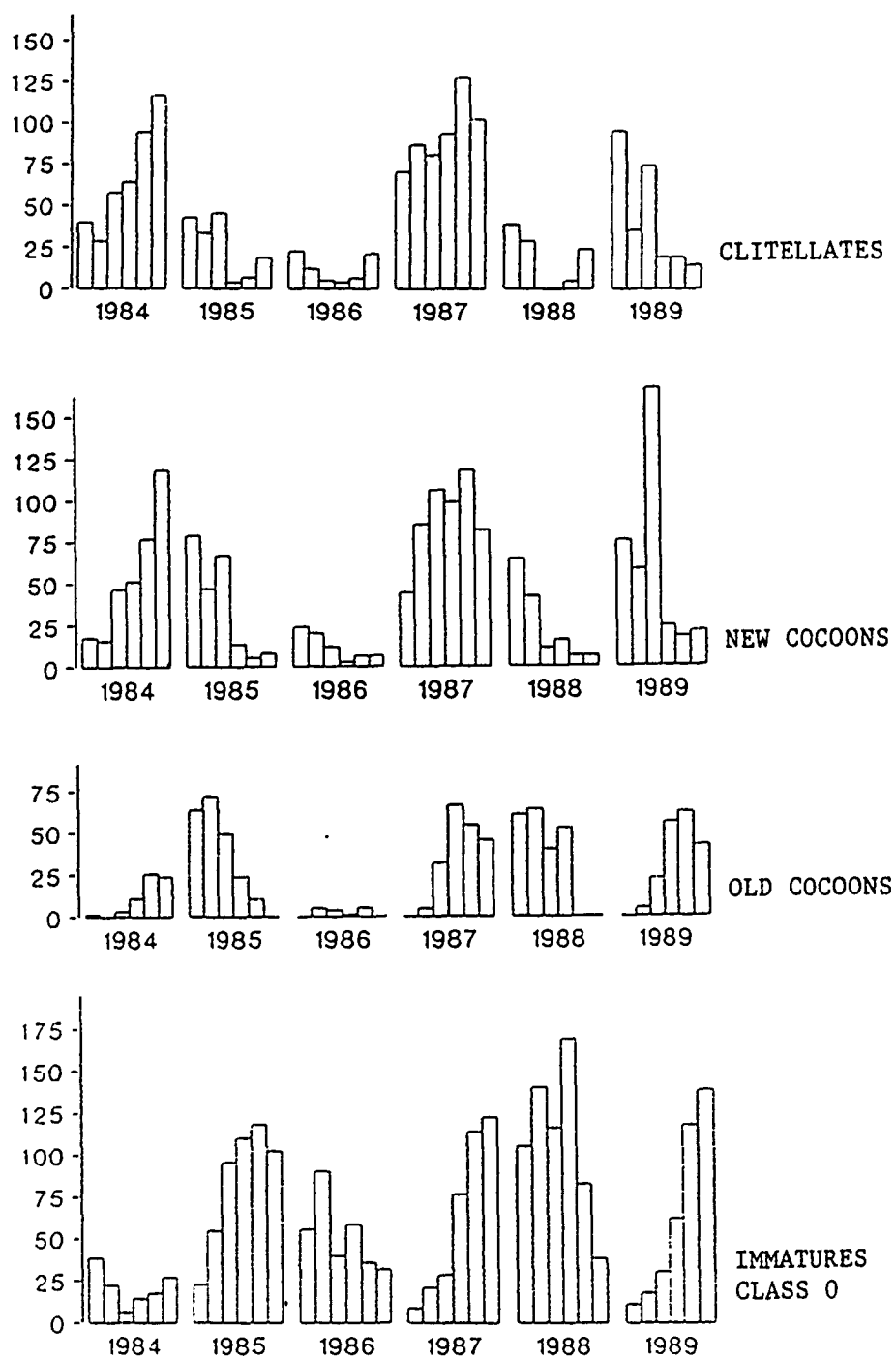
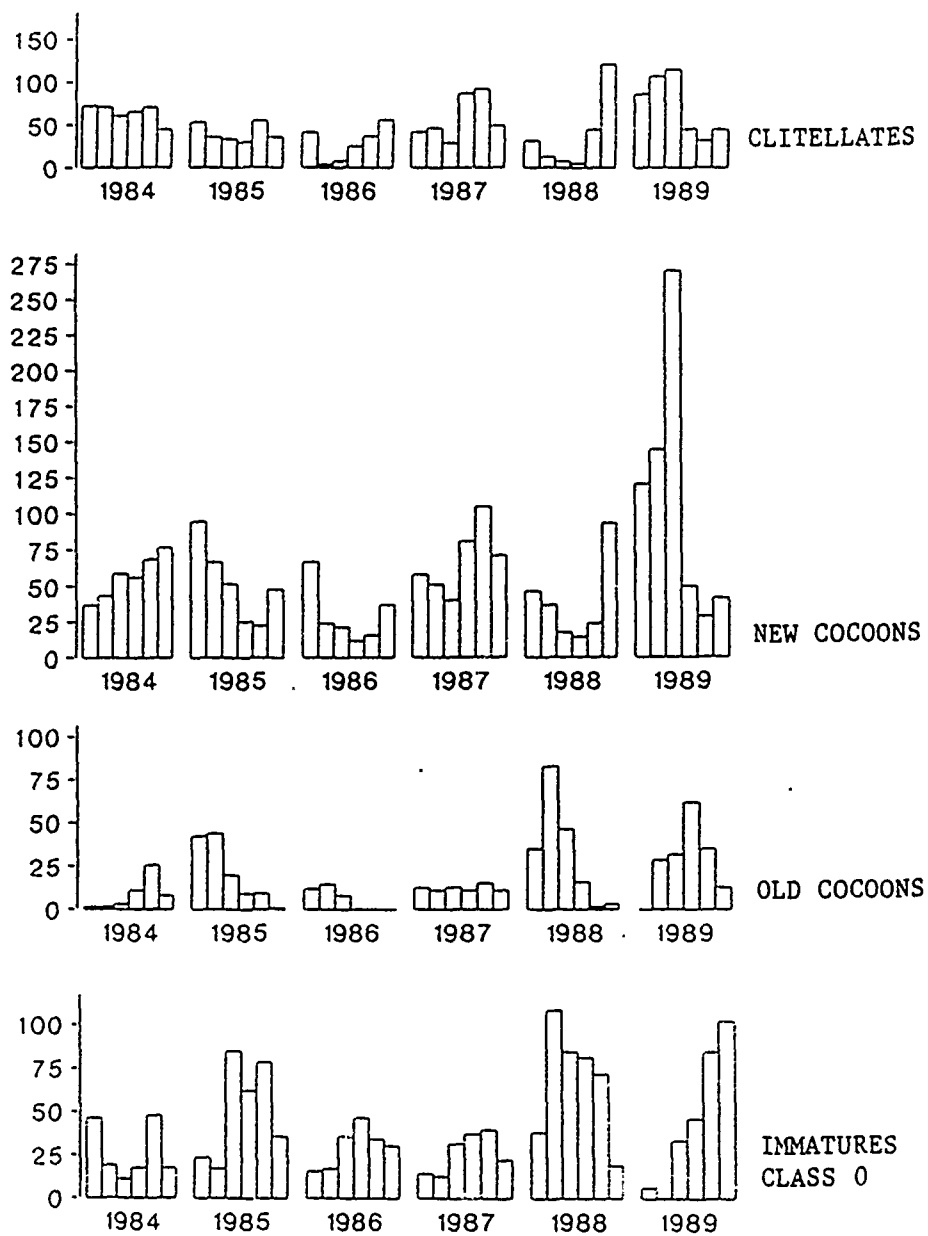Fig. 38. Mean monthly densities of clitellates, cocoons and hatchlings of A. tuberculata (Test), 1984-1989.

Fig. 39. Mean monthly densities of clitellates, cocoons and hatchlings of A. turgida (Control), 1984-1989.

drought years, when reproduction is suppressed, recruitment can still be substantial due to carry-over of the previous year's cocoons. Two special cases should here be pointed out:

a) Low cocoon production in the second half of 198⁻ and during all of 1986 should have reduced hatchling densities in 1986 to a noticeable degree, particularly in A. tuberculata. Recorded abundance of small immatures was, however, relatively high (Fig. 38). It is likely that under severe drought conditions, mass loss of individuals (which can be 50-60% of body weight) introduces some error: chronologically old individuals may then be classified as young immatures.

b) Mean new cocoon densities reached a single unprecedented peak in July of 1989. In both species (Figs. 38 and 39), this unusual mean was due to a single sample which contained 55% of all cocoons obtained on that date. In the following, whenever mean cocoon densities are used, this "aggregation" of cocoons will be excluded from the data.

It is obvious that timing and degree of reproductive activity are influenced by soil moisture in similar ways in both species. However, only one parameter has emerged as useful for comparing them: the mean annual percentage (of all adults) clitellate was significantly correlated between species (r = 0.85, P $<$ 0.05).

For each species separately, significant relationships between mean seasonal clitellate density and new cocoon density were obtained. Only data from dates 4 through 11 were used, to avoid biased cocoon estimates at times when low temperatures slow development and cocoons may be chronologically older than they appear. Regression coefficients (N = 6 years) were 0.97 for A. tuberculata and 0.95 for A. turgida. Differences in the persistence of clitellates during drought (higher in A. turgida) and

probably in the rate of cocoon production resulted, however, in differences in regression parameters.

To illustrate potential variability, lumped 1984-1988 data are opposed to 1989 regressions in Fig. 40. In both species, clitellate/cocoon relationships in 1989 deviated drastically (and in the same direction) from the long-term average.

Statistically, no clean relationship between moisture and reproduction parameters could be extricated from the data. Qualitatively, soil moisture in mid-summer (July) appeared to be a crucial factor. Lowest July moisture estimates were obtained for 1986 and 1988; coupled with prolonged drought, they curtailed reproduction in both species to the greatest degree (Figs. 38 and 39). Soil moisture was also low in July 1985, but the dry season was of short duration: intermediate clitellate and cocoon densities were recorded for both species (Figs. 38 and 39).

7.3. Population structure

Calculated on a monthly basis, densities of individuals in selected weight classes are shown in Figs. 41 and 42.

Peak hatchling abundance can occur at any time of the season, since it is a function of the variable timing of cocoon deposition. As a result, weight class frequencies vary from year to year, making detection of growth and maturation patterns difficult.

Although field-derived evidence is somewhat weak, we estimate that both species require 2 years (longer if drought delays growth) to reach maturity. Relatively high cocoon production by A. tuberculata in late 1984 and early 1985, for instance (Fig. 38), resulted in large numbers
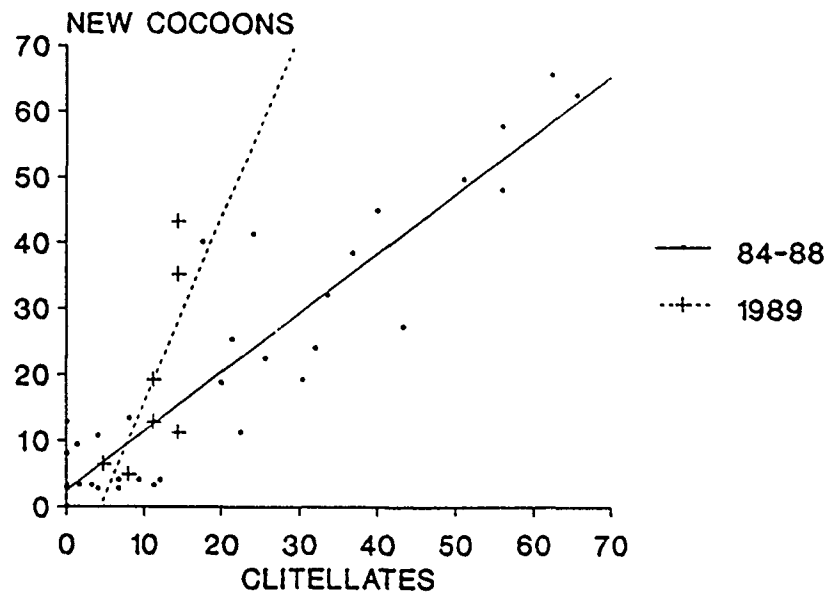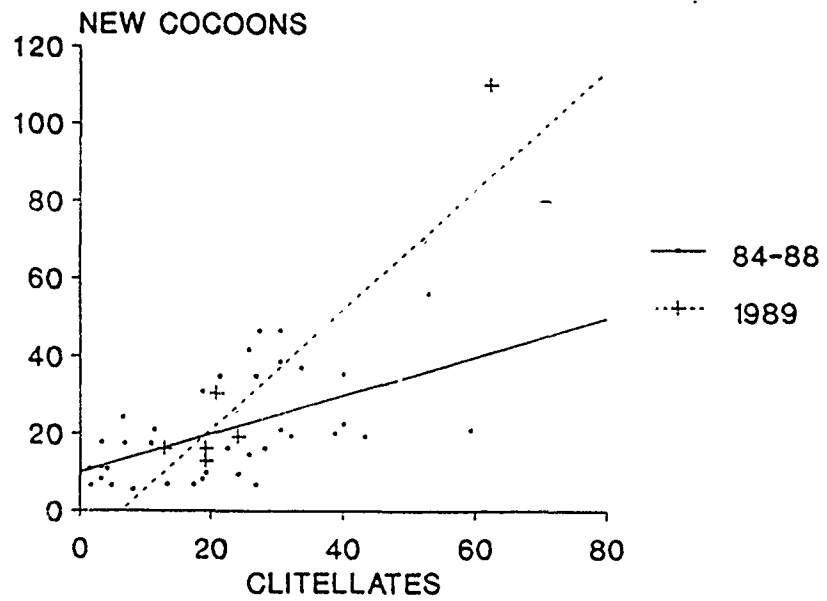
A. tuberculata

A. turgida

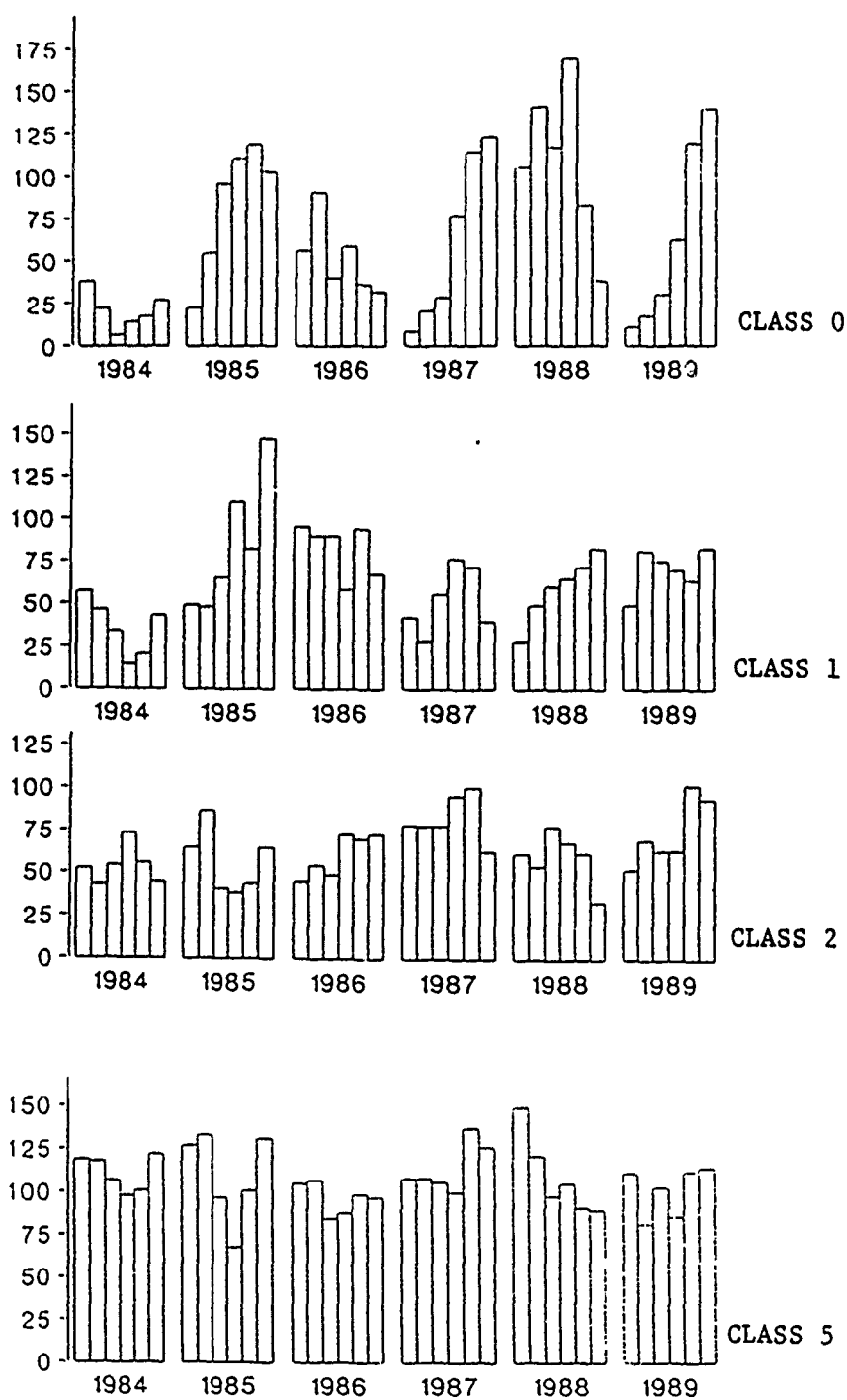Fig. 40. Regression of new cocoon on clitellate densities of A. turgida and A. tuberculata.

Fig. 41. Mean monthly abundance of selected weight classes of
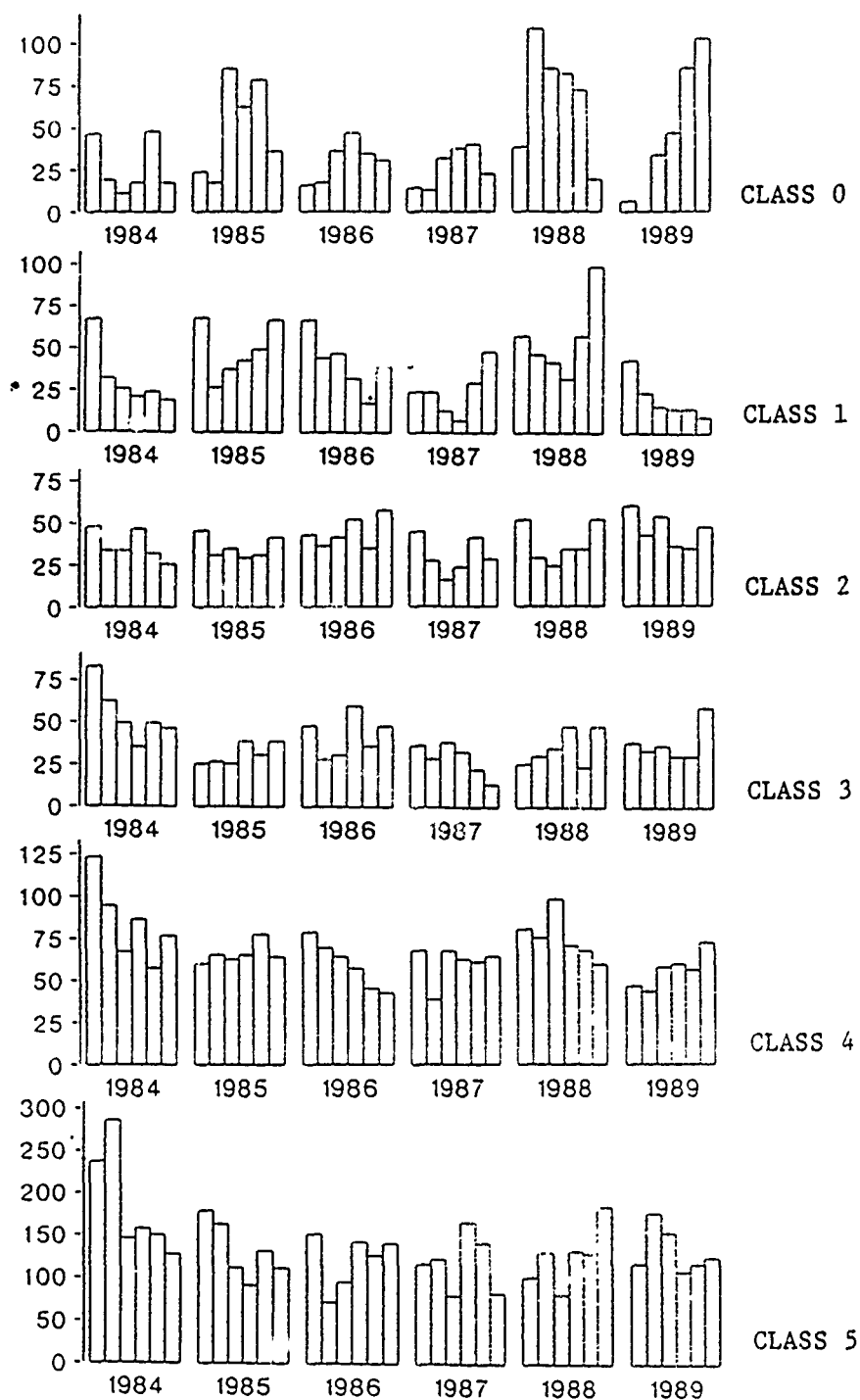A. tuberculata, 1984-1989 (for class identification see Table 14).

Fig. 42. Mean monthly abundance of weight classes of A. turgida, 1984-1989 (for weight class identification see Table 14).

of small immatures in 1985 and early 1986; increases (though indistinct) in class 5 immatures and adults did not occur, however, until 1987-88 (Fig. 41).

Temporal relations between cocoon deposition, development and hatching are clear, and parallel in A. turgida and A. tuberculata (Figs. 38 and 39). In both species, subsequent patterns of growth are blurred by: changes in body mass associated with dessication, hindering interpretation of weight classes in terms of chronological age; mortality rates, which are unknown; and variations in growth of different cohorts due to winter temperatures or seasonal droughts.

A condensed summary of weight class distributions is presented in Fig. 43, in which mean arnual abundance of selected population segments are shown. High total numbers of A. turgida in 1984, with a preponderance of large immatures and adults, are obviously the result of events which took place 2-3 years prior to the beginning of the study. Thereafter, A. tuberculata exhibited greater population stability, while A. turgida declined to a significant low in 1987. We suspect that the relative stability of the heavier-bodied A. tuberculata is due to greater longevity and to greater resistance to adverse moisture conditions.

Data on population structure are not suitable for rigorous comparisons between the species (and sites). They are useful, however, for monitoring long-term general trends. In both species, for instance, the highest influx of recruits occurred in 1988 (Fig. 43). As a result, we expect that abundance of large immatures and adults may noticeably increase by late 1990 and into 1991. If recruits stemming from 1989 cocoons (despite depressed reproduction in the second half of that year) further swell the population, total numbers may exhibit increases similar to 1988.
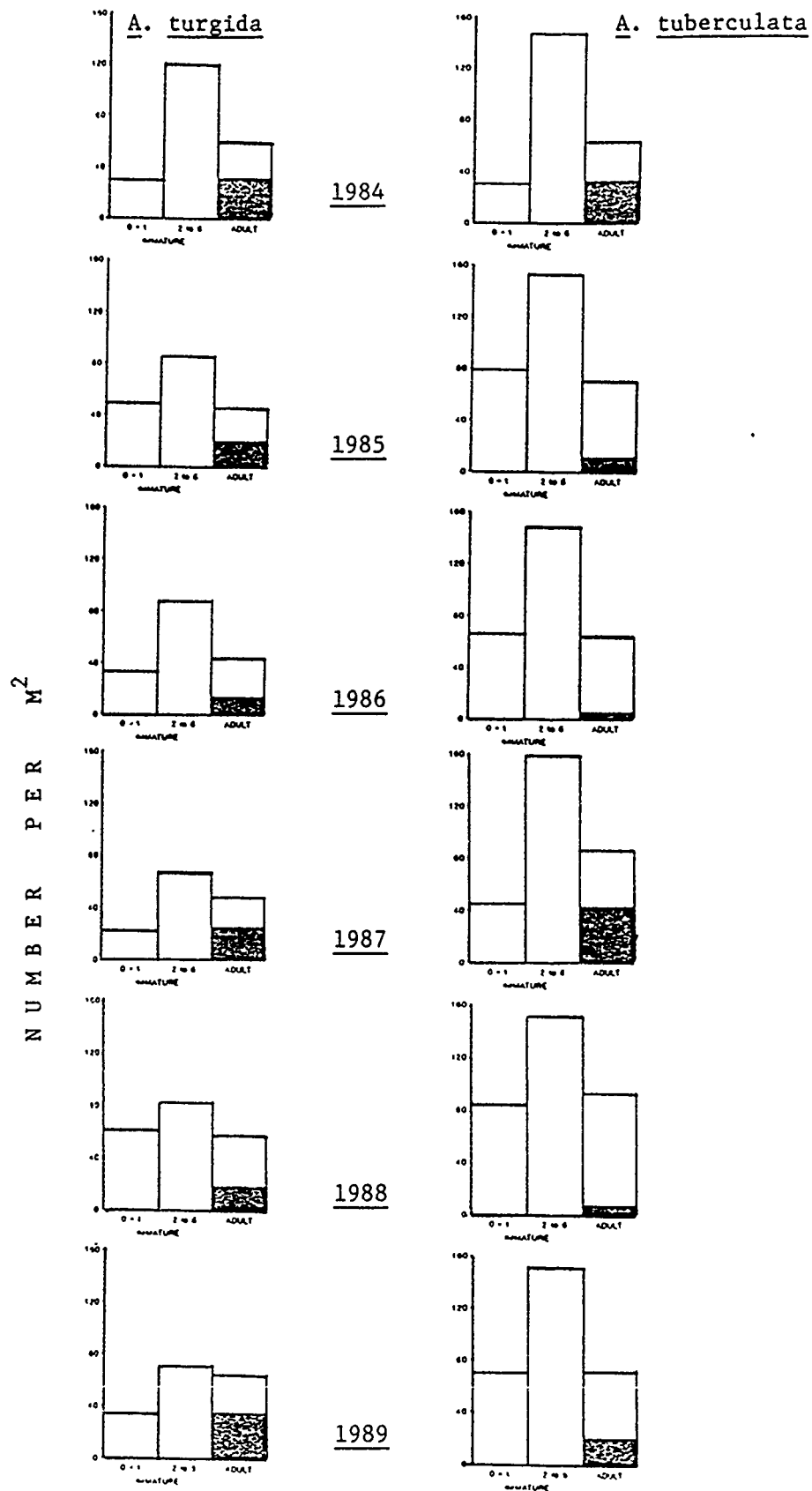
Fig. 43. Mean annual abundance of small immatures (class 0+1), larger immatures, and adults (black portion = clitellate density) of A. turgida and A. tuberculata, 1984-1989.

V. LITTER INPUTS AND DECOMPOSITION

1. Litter inputs

Litterfall patterns in 1989 were tightly synchronous in Test and Control (Fig. 44). In the past, total inputs/year tended to be slightly higher in Control; in 1989, Test litter inputs were slightly (again not significantly) higher in Test (Table 22). Data for both sites were entirely consistent with previous years.

Table 22. Yearly litter inputs (g dry / m^2), 1983-1989, by the dominant Acer saccharum and by all species together.

		1983	1984	1985	1986	1987	1988	1989
Maple	T	189	177	203	176	161	191	180
	C	221	179	199	189	180	198	162
Total	T	278	259	286	252	231	276	269
	C	305	264	289	284	275	301	258

2. Litter standing crops

Using oven-dry litter mass data, we have shown earlier that standing crops were lower in Test than in Control, particularly during mid-summer. Minima and maxima in late summer and fall were generally equal in Test and Control. As examples of between-year variability, Fig. 45 illustrates 1987 through 1989 data: in neither site were 1989 estimates discrepant.

In order to improve these estimates, we have ground and ashed litter samples of the 1987 through 1989 seasons (samples not available for earlier years). Control tended to have higher ash values than Test litter. Data
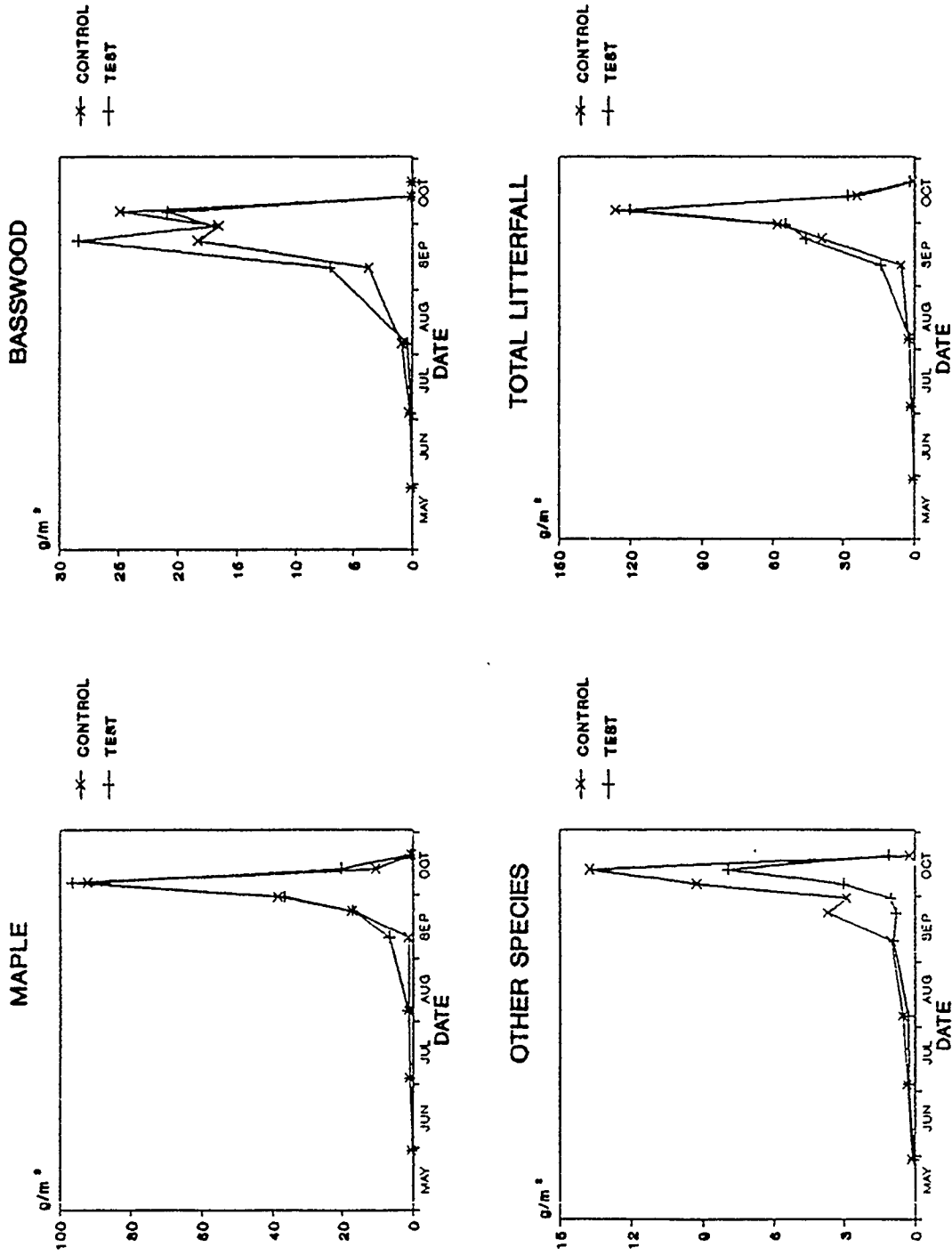
100

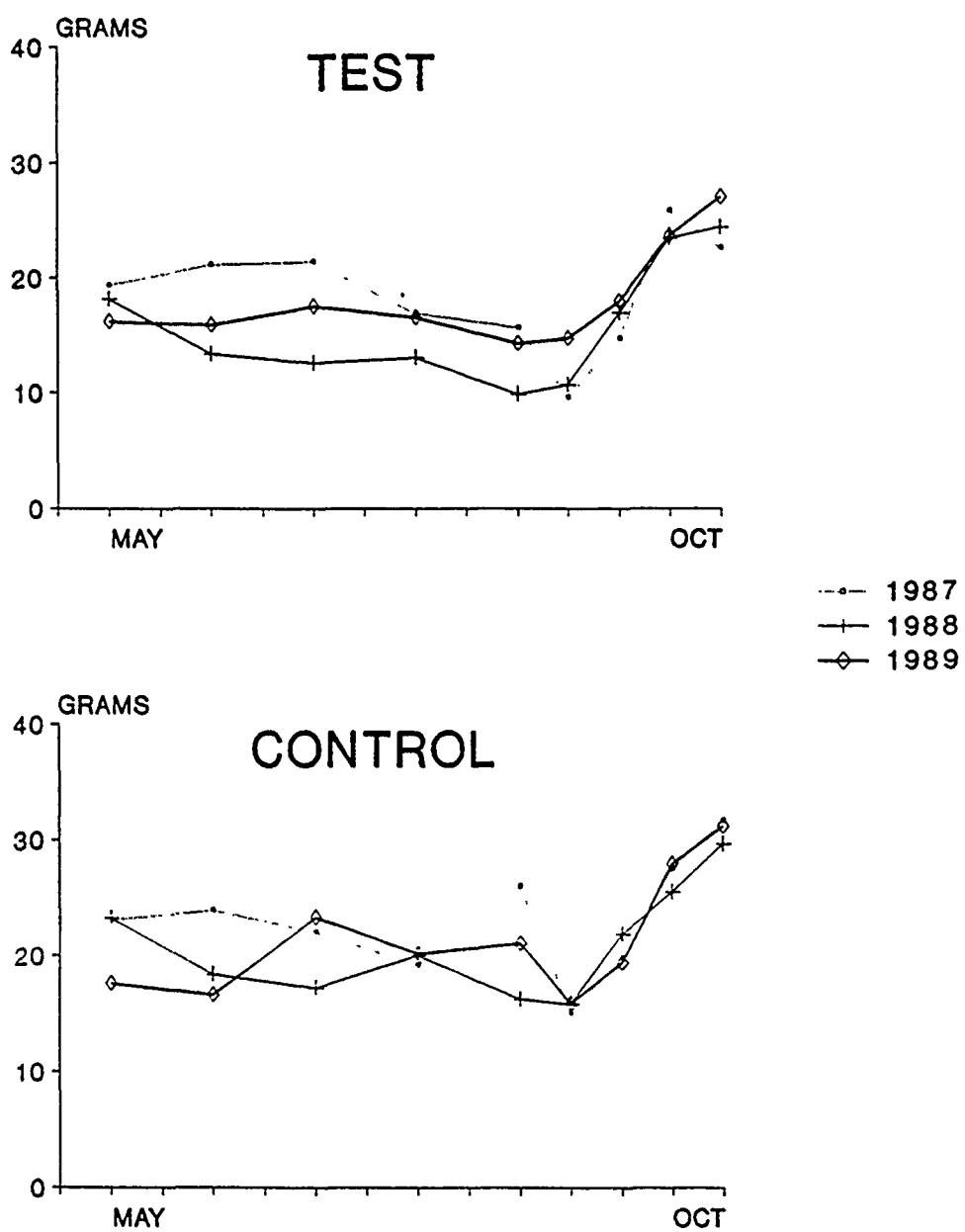Fig. 44. Seasonal litter inputs (g dry/ m^2) in Test and Control, 1989.

Fig. 45. Seasonal litter standing crop estimates (oven-dry,g/m^2) for Test and Control, 1987-1989.

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from the 1989 season (ash-free dry weights) are presented in Fig. 46, illustrating the reduction of variability and of between-site differences if AFDW is obtained.

The 40 replicates / date are obtained in pairs: two contiguous samples (one for moisture determination, the other for arthropod ectraction) are taken in each of the 20 quadrats/ site. Preliminary Anova showed that variation between quadrats was significantly higher than among sample pairs. We therefore used means of each pair (reducing N to 20/ site/ date) for further analysis.

Anova of seasonal AFDWs (1987 through 1989) showed that overall site effects were not significant; neither were site x date and site x year x date interactions, indicating that patterns of input and decomposition progress similarly in Test and Control. Years, dates, and their interaction were significant; while it is obvious that standing crops should vary with date, these results also indicate that some year-to-year variability can be expected.

Despite these statistical results, some real differences between sites may well exist. The litter stratum in Control appears less homogeneous than in Test, in scattered spots being of more complex structure. We are planning further analysis of long-term, quadrat-specific data bases of standing crops and macrodecomposer populations (lumbricids). Results may lead to further questions regarding the homogeneity of habitat and animal population distributions within each site.

3. Decomposition

3.1. Turnover time

We have reported turnover times of approximately 1 year for 1985
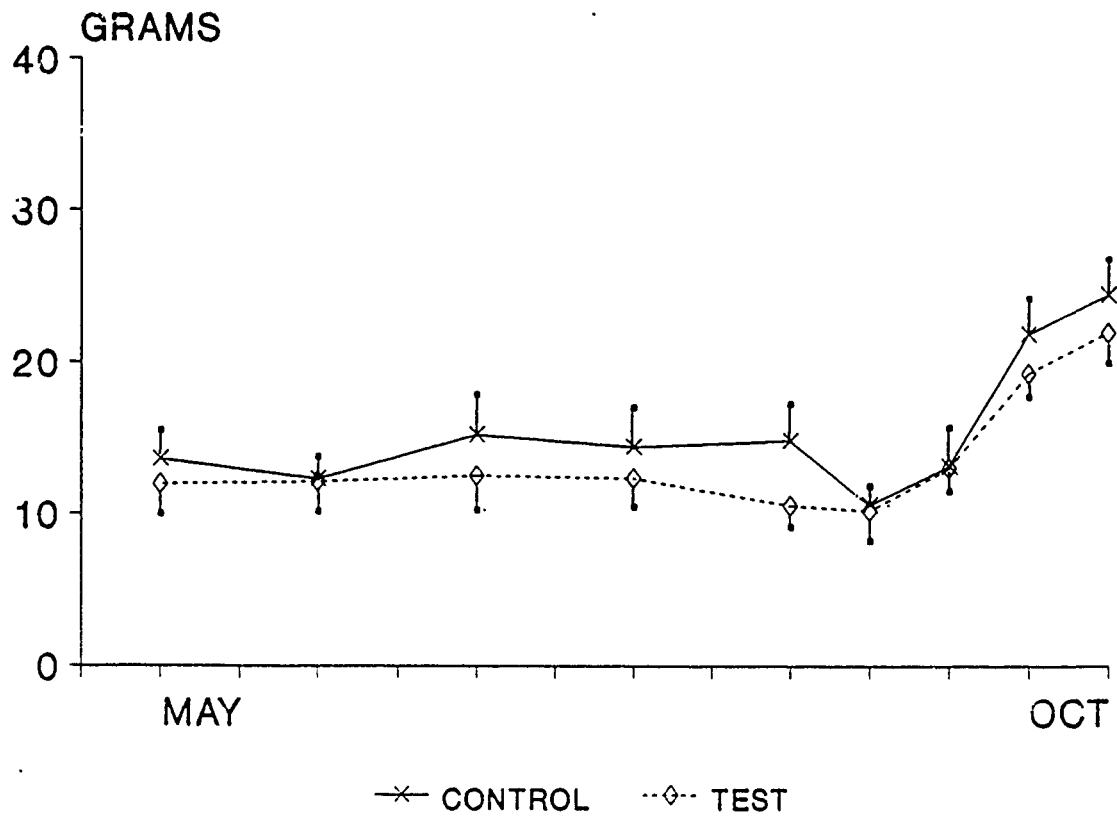
Fig. 46. Seasonal litter standing crops (AFDW, g/m^2), Test and Control, 1989.

and 1986. This parameter proved to be more variable than expected in subsequent years. Based on oven-dry weights, estimates varied between 0.82 and 1.22 years for Test, and between 0.97 and 1.37 years for Control (Table 23).

These values are only weakly correlated between sites. Turnover times for Control were higher than Test estimates in four of five years; however, 5-year averages (1.0 for Test and 1.1 for Control) did not differ significantly between sites.

Yearly variation in litter inputs and maximum standing crops obviously influences turnover time estimates. However, it is striking that the greatest discrepancy between sites occurred in 1989: a turnover time of 1.37 years for Control may be associated with an all-time low population of D. octaedra, a major litter consumer. More stable turnover estimates for Test could be related to relatively stable population densities of L. rubellus. We have not yet explored this link; future years' data may elucidate these questions.

Table 23. Turnover times for leaf litter in Test and Control, 1985-1989.

	1985	1986	1987	1988	1989
TEST	0.99	0.97	1.22	0.82	1.03
CONTROL	0.97	1.00	1.28	1.00	1.37

5-year means ± 95% CL: Test = 1.0 ± 0.19
Control = 1.1 ± 0.26

Note: Turnover time = $1/k$; $k = -\ln (1-k')$;
k' = input / max. standing crop

3.2. Litterbags

The litterbag series implemented in November 1988 was sampled through-
out 1989, at monthly intervals from May to November. These data can now
be compared with the first series of 20 mm mesh bags placed in the field
in November 1985. For both series, AFDW were obtained.

Fig. 47 shows all pertinent comparisons, beginning with 1986-87 data
for Test and Control, which did not differ significantly (Fig. 47 A).
First-year decay in Test did not differ between years, approximately
40% of initial mass remaining after one year in the field (Fig. 47 B).
Significant differences between sites occurred in October ($P < 0.01$)
and November ($P < 0.001$) of 1989, Control litterbags retaining more
mass than those in Test (Fig. 47C). Clearly, first-year decay rates
in Control were lower in 1989 than in 1986, differences again becoming
apparent in October and November (Fig. 47 D).

These results once more raise questions (ref. preceding section)
regarding the influence of site-specific biotic activity on litter
breakdown, with D. octaedra as a likely candidate. Tentatively, we
suggest that summer decay rates were not affected because earthworm
activity is suppressed by drought during most summers; in the fall,
when environmental conditions would have been propitious to D. octaedra,
large enough numbers were simply not present in 1989 to effect breakdown
in keeping with previous years.

Because litterbag data have proven relatively sensitive to changes,
we initiated an additional unplanned litterbag series in November 1989,
although there was not time enough to prepare enough replicates for two
full years of retrieval. The resulting revised schedule for monitoring
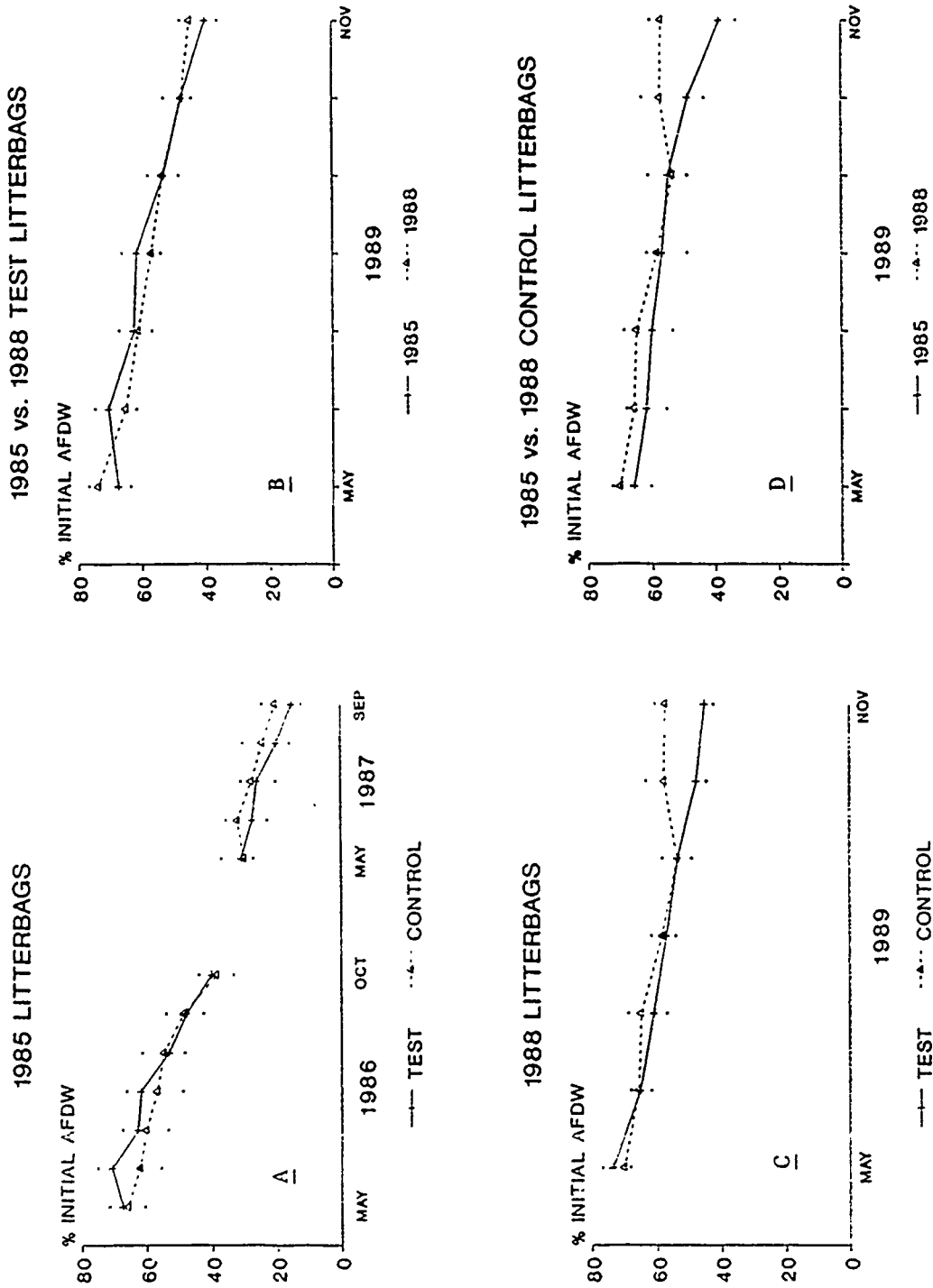
Fig. 47. Percent of initial weight remaining in Test and Control litterbags of two series, one initiated November 1985, the second November 1988 (means ± 95% CL).

litter decay during the critical ELF years is given in Fig. 48. The pre-
viously proposed schedule (Fig. 49) included second-year data through
November 1992. Since 1989 may (by some) not be considered a fully opera-
tional year, we believe that additional first-year decay data should
be obtained for all three operational years available to us (seasons
of 1990, 1991 and 1992). As shown in Fig. 48, a last EXCHANGE series
(Test litter in Control and vice versa) may be initiated if 1991 data
indicate the necessity for it.

L I T T E R B A G S

Nov 1988	Nov 1989	Nov 1990	Nov 1991	Nov 1992
SERIES I 1st yr decay	SERIES I 2nd yr decay			
	SERIES II 1st yr decay			
		SERIES III 1st yr decay	SERIES III 2nd yr decay	
		SERIES IV EXCHANGE 1st yr decay	‾ERIES IV EXCHANGE 2nd yr decay	
			SERIES V 1st yr decay	
			SERIES VI ? EXCHANGE ? 1st yr decay	
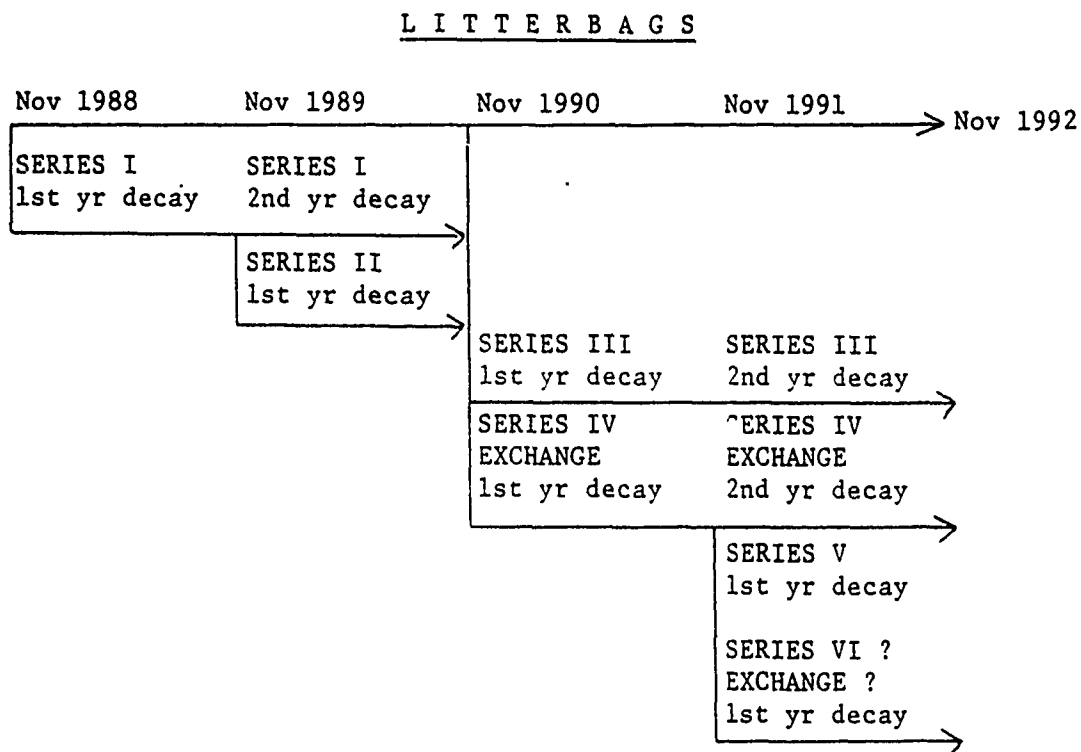
Fig. 48.Litterbag study schedule proposed for the remaining project years
(series I, second year, and series II, first year, to be sampled in the
current 1990 season).

L I T T E R B A G S

Nov 1988   Nov 1989   Nov 1990   Nov 1991
                                                              → Nov 1992

SERIES I   SERIES I
1st yr decay   2nd yr decay

                               SERIES II   SERIES II
                               1st yr decay   2nd yr decay

                               SERIES III   SERIES III
                               EXCHANGE   EXCHANGE
                               1st yr decay   2nd yr decay

Fig. 49. Litterbag study schedule as proposed in 1987.

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ELF Communications System Ecological Monitoring Program

BIOLOGICAL STUDIES ON POLLINATING INSECTS: MEGACHILID BEES

Annual Report 1989

Karen Strickler
J. Mark Scriber
Department of Entomology

FRONTISPAGE

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Reporting year: 11/1/88 - 10/31/89

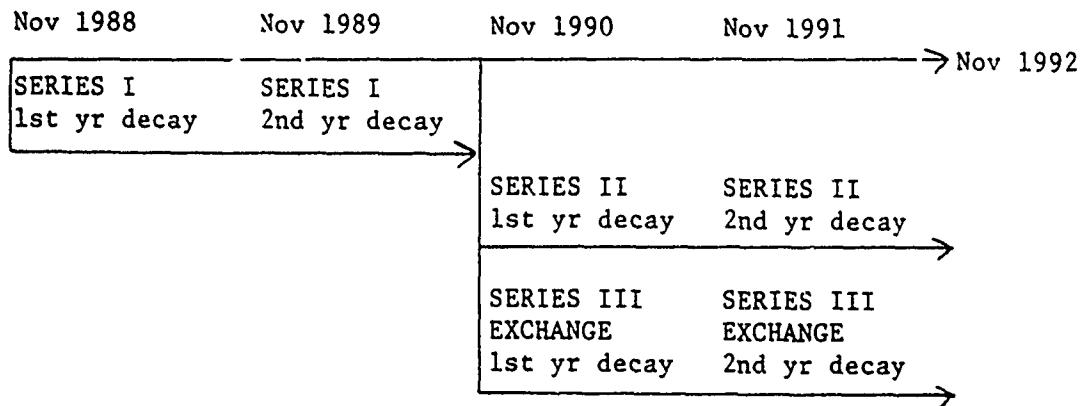Prepared by: Karen Strickler, Assistant Professor
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LIST OF ACRONYMS

C5:	Camp 5 control site
CATMOD:	Categorical data modeling procedure in SAS
CL:	County Line control site
ELF:	Extremely Low Frequency
EM:	Electromagnetic
Exp:	Variable indicating whether the data were from an experimental or a control area.
Exp*Year:	Interaction effect of the Exp and year variables in the GLM, ANOVA, or CATMOD model.
F1:	Ford 1 (north Ford) experimental site
F2:	Ford 2 (south Ford) experimental site
GLM:	General Linear Modeling procedure in SAS
LO:	A round leaf piece used to cap a cell, plug a nest, or occasionally at the base of a cell. Occasionally an LO will be part of the construction of a cell lining as well as LRs. The bee carries an LO in her mandibles.
LR:	An elongate, oblong leaf piece used to line a cell. The bee carries an LR rolled between her legs.
Measurer:	variable indicating the observer or measurer of data.
SAS:	Statistical software package on the VAX computer, used in analysis of data
Site [exp]:	Site variable nested in experimental areas
Trip Rank:	The number of LO leaves already collected by a bee, including the current LO leaf, in a series of LO trips to cap a cell. Usually the duration of the first 5 such trips are recorded for a given cell cap. These LO trip durations are given Trip Ranks of 1, 2, 3, 4, and 5.
Yr:	Year

I. ABSTRACT

High voltage transmission lines and magnetic fields have been shown to affect honeybee reproduction, survival, orientation, and nest structure. ELF EM fields could have similar effects on native megachilid bees.

Two species in the genus Megachile have been most abundant in artificial nests at experimental and control sites in Dickinson and Iron Counties. Data on their nest architecture, nest activity, and emergence/mortality have been collected since 1983. Five hypotheses concerning the possible effects of ELF EM fields are considered using these data. Although the ELF EM fields were at 100% power for most of the summer of 1989, in this report only nest activity data for 1989 reflects this exposure. Nest architecture and mortality for nests constructed in 1989 will not be analyzed until 1990. Nests created in 1988 were exposed to 50% ELF EM fields during both summer and winter.

Thus far, we have not detected significant differences between experimental and control areas in cell lengths, number of cells per nest, number of leaves per cell, orientation of nest entrances, or time to collect a leaf to cap a cell. Furthermore, there are no significant differences in the interaction between year and experimental vs. control areas in these factors.

Sample sizes similar to those obtained in 1987 - 1989 should be sufficient to detect reasonable differences (9 -15%) between experimental and control areas in cell lengths and leaves per cell. A 1.9 fold change in time to collect a leaf for a cell cap (from 22 to 41 seconds) and at least a 3 fold change in overwintering mortality (from 5% to 15%) are needed before we can detect statistical differences between experimental and control areas. Changes of this magnitude are possible, especially considering the low means for these variables, and are worth continued monitoring efforts.

One possible effect of ELF EM fields was detected in this year's analysis. M. inermis prepupal (overwintering) mortality in nests oriented along a NS axis was lower in experimental than in control areas for 1988 nests. This was the first year with significant testing of the antenna during the winter, and the first year that the nests were overwintered in the direction that they were constructed. We will be interested to see if this pattern is repeated in the next few years.

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II. INTRODUCTION

Project Rationale and Overall Objectives.

High voltage transmission lines and fluctuations in the earth's magnetic field have been reported to affect honeybees (Greenberg et al. 1981; Gould 1980). In addition, honeybees have been shown to have an organ in the abdomen consisting of magnetite particles that could be used to detect the earth's magnetic field and thus could be used as a compass in orientation (Gould et al. 1978). In addition, this organ appears to be involved in the detection by foraging honeybees of localized magnetic anomalies associated with nectar rewards (Walker and Bitterman, 1989). Because such effects of electric and magnetic fields have been demonstrated, it is possible that ELF EM fields may alter a bee's ability to orient or may otherwise affect its behavior.

Honeybees, however, are rare in the state forest where the Michigan ELF antenna is located, and are unable to overwinter in the harsh climate of Michigan's Upper Peninsula (Fischer, 1983 Annual Report). Therefore, native bees are a better choice for ecological studies of the resident bee fauna. Native bees are particularly important in ecological communities such as those in the vicinity of the ELF antenna because they are pollinators of flowering plants, and are therefore important to the reproductive success of these plants.

With the exception of bumblebees and some halictids, native bees are solitary, meaning that each female constructs and provisions her own nest rather than having a special queen caste responsible for reproduction. Solitary bees have several advantages for ecological studies. As "mass provisioners", they create a discrete cell for each offspring, and fill it with a provision mass of pollen and nectar prior to laying the egg. The bee does not add more provisions after the egg is laid. A series of such cells, each with a provision mass and egg, are created in succession by each female. The provisions that go into each cell are a direct measure of parental investment in an offspring (Strickler, 1979). The size of the adult bee that emerges from each cell is correlated with the amount of provisions provided it, and with the size of the cell in which the larva develops (Krombein 1967; Klostermeyer et al. 1973; Torchio and Tepedino 1980; Alcock 1979). However, there is a tradeoff between the investment per offspring and the rate at which offspring are produced. The more the bee invests per offspring (ie, the larger the offspring), the fewer offspring she will produce. If bees are disoriented, agitated, or slower at foraging, they may invest less per offspring, produce fewer offspring per unit time, or both. Solitary bees are unusual in having this direct relationship between parental investment per offspring, adult size, and reproductive output.

The nesting biology of some species of solitary bees in the family Megachilidae is especially easy to study because they accept artificial nests placed in the field. These bees typically nest in abandoned beetle bores in dead logs. "Trap nests" of drilled blocks of wood are also used by bees as nest sites. Such artificial nests can be placed in habitats where bees are expected to nest, in order to increase the sample of nests available for study, and to standardize such characteristics of the nest as bore depth and diameter (Krombein, 1967). Trap nests are used in the management of the leafcutter bee, Megachile rotundata, for pollination of alfalfa (Stephen, 1962; Bohart and Knowlton, 1964; Johansen et al., 1969; Gerber and Klostermeyer, 1972; Hobbs, 1972). Thus there is an extensive (though unreviewed) literature on megachilid biology.

Research on the effects of high tension wires and magnetic fields on honeybees suggests working hypotheses on which to base our initial analyses of megachilid nesting biology. Of possible relevance to megachilid behavior are an alleged greater tendency for dispersal, and greater levels of activity (Wellenstein, 1973), as well as reduced reproductive output, lower overwintering survival, and modifications of nest structure (Greenberg et al., 1981) when colonies were exposed to electromagnetic fields from high voltage transmission lines. Disturbance of colonies under transmission lines can be attributed principally to electric shock from induced hive currents, especially under wet conditions (Bindokas et al. 1988). In addition, disorientation due to fluctuations in ELF magnetic fields is possible if megachilids share the honeybee's ability to detect magnetic fields. (Gould et al., 1978, 1980; Gould 1980; Tomlinson et al. 1981; Walker and Bitterman, 1989).

Nesting Biology of Megachilid Bees

A decision to restrict our study to two species of leaf-cutter bees, Megachile (Megachile) relativa Cresson and Megachile (Megachile) inermis Provancher, was made in the fall of 1986 (1986 Annual Report). M. inermis and M. relativa have similar nest architecture in that both line their cells with pieces of cut leaves. However, the two species differ in size, and may therefore partition their time and the space in their nests differently. Aspects of the biology of both species have been described for populations in Wisconsin and Canada (Medler, 1958; Medler & Koerber, 1958; Stephen, 1955, 1956; Longair, 1981).

The general structure of the nests of the two species is depicted in Fig. 1. The bee may leave some space at the base of the nest (the basal space) unoccupied by cells for offspring. She may then cut and bring to the nest a few round pieces of leaf which are added one at a time to form the base of the first cell. Next she cuts and brings to the nest several elongate pieces of leaf (LRs) in succession. These are used to line a tube- or cup-shaped cell that is slightly longer than her body. Next she makes a series of pollen and nectar foraging

trips to fill the cell with the discrete provision mass that will be the larva's food supply. When provisioning is complete, the female lays an egg. Fertilized eggs become females while unfertilized eggs become males. The female has voluntary control over fertilization and thus the sex of the offspring in each cell (Klostermeyer and Gerber, 1970). After laying the egg, she cuts more leaves, this time round in shape (LOs), to cap the cell. Somet'nes she adds chewed leaves, sand, or bits of wood to separate the cells. Next she cuts more elongate leaves for the second cell, and repeats the process. Thus a linear series of cells is constructed in the nest bore. Typically, the cells at the base of the nest are more likely to contain females and the cells near the entrance are more likely to contain males (Krombein, 1967). Since females are usually larger than males in these bees, cells at the base of the nest tend to be larger than cells near the entrance. When she has completed the last cell that she is going to put in the nest, she constructs a series of plugs of round leaves, chewed leaves, dirt, chewed wood, and possibly other material. M. relativa frequently includes empty "vestibular" spaces between segments of plug. M. inermis and some M. relativa create one long mass of plug material after completing the reproductive cells. In nests of both species there may also be space between the outermost plug and the opening of the nest, called an "indentation".

Each female may construct several such nests over her life time. Some nests are abandoned before they are finished because the bee has died, or for other unknown reasons. The adult life span is no more than one season; adults do not overwinter.

Inside each cell the egg hatches, and the young larva feeds on the provisions prepared by its mother. Both Megachile species in our study are univoltine in Northern Michigan (with a few rare exceptions; see Emergence Results), and both overwinter as prepupae. Pupation occurs in Spring, and adults emerge soon after, in June and July at our study sites. A variety of parasites may emerge from the cell instead of the original bee. Oviposition of parasite eggs usually occurs while the cell is being provisioned, when the mother bee is out of the nest on a pollen foraging trip, or on a round-leaf foraging trip just after laying her egg.

Hypotheses Tested

During the first four years of the project, 1983-1986, data on nest architecture, nest orientation, emergence/ mortality and nest activity were collected. Based on these data, six tentative hypotheses concerning the effects of ELF EM fields on Megachile behavior were specified in the 1986 Annual Report. The initial hypotheses were modified in previous reports based on our ability to gather sufficient sample sizes to detect differences between experimental and control areas. The modified hypotheses are expressed in the following sections as null hypotheses, ie., hypotheses of no difference

between experimental and control areas, that we will try to disprove statistically. The "Rationale" sections explain the possible effects of ELF EM fields that may cause a rejection of the null hypothesis.

Hypotheses Involving Nest Architecture:

Hypothesis 1: The average size (length and volume) of cells for each offspring, and/or the average number of cells produced per nest is unchanged by exposure to ELF electromagnetic fields.

Rationale

Honeybee reproductive output decreased on exposure to high voltage transmission lines. Capped brood, which normally averaged 12,000 per hive, decreased to as low as no brood after 8 weeks of exposure (Greenberg, et al., 1981). ELF EM fields may have a similar effect on the number of cells produced by megachilids. Furthermore, ELF electromagnetic fields may affect cell size and nest architecture in various ways. For example, if bees are disoriented by the fields, they may gather resources (leaves, pollen) more slowly when exposed to the fields than when not exposed. As a result, they may produce new cells at a slower rate, or they may produce smaller cells.

Previous studies have found that the weight of offspring of the generalist megachilid, Osmia lignaria, is lower if their cells were produced late in the season rather than early in the season (Torchio and Tepedino, 1980). This species also showed an increase in the proportion of male offspring (the smaller sex) produced late in the season. A reduction in offspring size late in the season is thought to be related to reduced foraging rates due to aging of the bee (Torchio and Tepedino, 1980, Tepedino and Torchio, 1982). Similarly, ELF EM fields may slow the foraging of M. relativa and M. inermis, resulting in smaller bees produced in smaller cells. A size reduction could affect cells with offspring of both sexes, or it could reflect the production of a greater proportion of male offspring, since males are the smaller sex in both Megachile species. An additional complication is that female sizes decrease more than male sizes late in the season (Torchio and Tepedino, 1980). Thus we might expect female cells to be affected more than male cells by stresses from ELF EM fields.

In contrast to the generalist megachilids, the pollen specialist Hoplitis anthocopoides did not show a reduction in offspring weight late in the season, in spite of reduced foraging rates (Strickler, 1982). Rather, it was hypothesized that slower foraging rates led to fewer offspring per nest late in the season as compared with early in the season for this species. Similarly, M. relativa and M. inermis may produce fewer cells per nest in response to slow foraging rates due to ELF EM fields.

In testing hypothesis 1 we are interested in determining whether there are differences between experimental and co:· · sites in cell lengths, cell volumes, and number of cells per nest. Ideally, we hope to find no differences between experimental and control sites, and between years, prior to the 1987 season when the ELF antenna was operational at low power. Then, if significant differences between experimental and control sites appear in the years after the antenna is turned on, we can attribute these differences to the effect of ELF EM fields.

Hypothesis 2: Bees exposed to ELF EM fields, and bees not exposed, will make nest plugs of the same thickness and will devote the same proportion of nest space to reproduction.

Rationale

Abnormal deposits of up to 48g of propolis were present at honeybee hive entrances under high voltage transmission lines, presumably in response to stress connected with electric fields at the nest entrance (Greenberg et al, 1981). This suggests the possibility that megachilid bees will respond to disturbance from ELF EM fields by increasing the amount of nest lining material in the bores. This ·..ay be reflected in larger cells (tested in hypothesis 1) and/or increased nest plug length. More generally, there could be an increase in the nest space that does not include cells for offspring (ie. basal and vestibular spaces, nest plugs and indentations).

Hypothesis 3. The number of leaves used to line a cell is unchanged when bees are exposed to ELF EM fields.

Rationale

Bees may pad a cell with extra leaves as a result of stress due to electromagnetic fields (see hypothesis 2). Originally we had planned to test this hypothesis using nest activity data, by counting the number of elongate leaf (LR) collecting trips taken by a nesting bee. However, in the 1986 Annual Report we concluded that the time available to test this hypothesis by watching bee activity would not yield sufficiently large sample sizes to detect differences between experimental and control areas. Instead, we proposed at that time to determine the number of elongate leaves used to line a cell by taking the cell apart after bee emergence.

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Hypothesis 4. The relative acceptability of nests oriented in a NS direction vs. nests oriented in an EW direction does not change when bees are exposed to ELF EM fields.

Rationale

Honeybees may use the earth's magnetic field under special circumstances to orient their comb (reviewed in Gould, 1980). The fluctuating ELF magnetic fields could disturb any biases that megachilids normally have for nest orientation, or could cause greater acceptance of nes · oriented in certain directions in order to reduce disturbance by the fields.

Hypotheses Involving Nest Activity

Hypothesis 5. The duration of round leaf (LO) foraging trips remains the same when bees are exposed to ELF EM fields.

Rationale

Honeybee activity, measured by honey production, allegedly doubled under high voltage electromagnetic fields in one study (Wellenstein, 1973). In contrast, colony weight, a measure of rate of honey accumulation and brood production, decreased by as much as half for colonies exposed to high voltage transmission lines in a different study (Greenberg et al., 1981). Honeybees also had an increased tendency to sting under high voltage transmission lines (Wellenstein, 1973). ELF EM fields might similarly affect megachilid bee activity by disorienting or agitating the bees so that the duration of leaf- and pollen-foraging trips is altered. Interference with magnetoreception might play a role in disorientation. Changes in electric potential of the bees, or of the plants on which they forage (Erickson, 1975) might also affect the bees' foraging rate.

Leaf-foraging trips for M. inermis are easy to recognize behaviors, usually lasting less than a minute in duration. Many of these trips are taken in succession, so within and between bee variability can be analyzed, and a potentially large sample of leaf collecting trips can be timed. In the 1986 Annual Report we demonstrated that the collection of LO leaves was the most consistent behavior of the leaf-cutting bees under study. We argued that this is probably because it is adaptive to close the cell as quickly as possible after the egg is laid to avoid parasitism. Thus, our analysis focuses on LO trip durations.

Hypotheses Involving Emergence

Hypothesis 6. Overwintering mortality of megachilid bees is unchanged by exposure to ELF EM fields.

Rationale

Overwintering mortality of honeybee colonies under high voltage transmission lines increases from 29% when hives were shielded to 71% when they were fully exposed to electrical fields. (Greenberg et al., 1981). We would like to test for a similar effect in megachilid bees. To do this requires comparing control and experimental sites in the proportion of cells that suffer mortality during the prepupal (overwintering) stage, relative to the number of cells that survive to the prepupal stage or beyond (pupa and adult) (see results section for further explanation).

According to Brodeur (1989, p.58), studies of the effects of ELF EM fields on chicken embryos suggest that teratological effects depend on the orientation of the embryo relative to both an artificially pulsed field and the earth's magnetic field. I have not found an original reference for this result. However if true, this suggests that overwintering mortality (as well as mortality of eggs and developing larvae) may be different for nests oriented in a north-south vs. nests oriented in an east-west direction.

III. METHODS AND TYPES OF DATA COLLECTED

Nest architecture and nest orientation are obtained by placing trap nests in the environment, and allowing bees to construct nests in their choice of traps during the summer. The following spring, various parameters of their nest architecture are measured. Bee and parasite emergence and larval and pupal mortality are also recorded in the Spring. Nest activity data are gathered during the summer season while the bees are constructing their nests.

The methods discussed below will compare, where appropriate, changes in protocol over the years, especially pre- and post-1987. Where no such comparisons have been made, no significant changes in protocol have been made.

Trap Nesting Methodology

Trap nests consist of elongate white pine pieces 19x19x153 mm. Most of these nests were drilled lengthwise to a depth of 142mm. Exceptions were the largest diameter pre-1987, and half of the 1987 large diameter nests. These nests were only drilled to 107mm.

Prior to 1987, six different bore "sizes" corresponding to the diameters of seven different drill bits, were used (Table 1). Two different drill bit sizes were used at various times for bore size 4. Note that the bore diameters are not associated with consecutive bore sizes. The maximum bore size was limited by the dimensions of the trap nest, and by availability of long drill bits.

In 1987 only bore sizes 4 (the 5.5mm bit) and 7 (11.0mm) were used because these sizes were accepted most often by the two Megachile species under study in 1985 (See 1986 annual report). In 1988 and 1989 bore size 4 nests were made with both 5.5 and 6.0mm drill bits because analysis of 1986 nests indicated that the 6.0 mm diameters were common, and because it was feared that 5.5 mm bores would skew the sex ratio in favor of male offspring and thus bias the cells towards shorter lengths. Bore diameter has been shown to influence sex ratio for other trap nesting species (Stephen and Osgood, 1965; Krombein, 1967).

Bores made with the same drill bit can vary in the final diameter of the nest due to variability in the drilling process, and to swelling or shrinking from weather. Because of this, and because the diameter of bore sizes has been changing over the years, our analyses are based on the actual measured diameter of the nest, rather than on the bore size.

Although M. inermis might prefer a bore size greater than size 7, we suspect that the preferred bore size may be intermediate between the two largest sizes provided. No bore diameters between bore size 3 (9.4mm) and 7 (11mm) were available. In the absence of such an intermediate size, the larger size was most frequently accepted. One observation that supports this postulate is the number of leaves used per cell (1987 Annual Report, Table 29), which increased with nest diameter for M. relativa from an average of 6.7 in bore size 4 to 10.3 in bore size 5. Similarly, in bore size 3 nests, M. inermis uses an average of 9.6 leaves per cell, whereas in bore size 7 nests, 13.1 leaves per cell are used. The additional leaves in large bore sizes may represent an attempt by the mother bee to pad a cell with a greater than optimal diameter. Our 1986 decision to use only bore size 7 nests was based on an attempt to maximize consistency between years, and to reduce variability due to nest diameter, rather than to choose a most preferred bore size.

In 1985 and 1986, twelve nests, two of each bore diameter, were bound together with plastic strapping into a "block", so that one of each bore size faced each direction, and no two bore entrances were adjoining (Fig. 2). Starting in 1987, two bore size 7 and four bore size 4 nests were arranged randomly in each direction (Fig. 3). (In 1988 and 1989, three of the small nests were 5.5mm and one was 6.0mm.) We did not realize that the 1987-89 random arrangement of nest entrances differed from the 1985-86 pattern of no adjoining entrances until blocks for 1987 had already been prepared. However, we do not believe that this change in nest arrangement affected the bee's behavior.

"Hutches" consisting of a wooden frame with four shelves and a roof were used to hold the blocks of trap nests (Fig. 4). Four blocks of nests were placed randomly on each shelf, making a total of 192 nests present at any one time. The hutch was open on both sides, so half of the nests opened in each direction. The shelves were roughly 0.1, 0.4, 0.8, and 1.1 meters from the ground.

Four study sites were selected in 1984 for placement of hutches. Two are experimental sites along the ELF antenna: Ford 1 and Ford 2 (F1 and F2), and two are control sites: Camp 5 and County Line (C5 and CL). The study sites are described below. Further information can be found in the 1985 annual report. Three sets of two hutches, making a total of six hutches, were placed at each of the four study sites. In each set of two hutches, one hutch was oriented in a north-south direction so that its nests open to the east or west, and one hutch was oriented in an east-west direction so that its nests open to the north or south. The two hutches in each set were placed close together in edge habitats between open areas where there are abundant flowering plants, and woods where natural nest sites are available.

When a nest was occupied by a megachilid bee, it was given a number that included site (C5, CL, F1, or F2), hutch direction (NS or EW), nest entrance orientation (E, W, N, or S) and shelf height (1-4, top to bottom). This number was written on the side of the nest. Position on the shelf and in the block of nests was not recorded. Starting in 1987, a computer data base was created to help us manage nest numbers and progress of the nesting bees.

Once a nest in progress was identified, the depth of empty tunnel space was recorded daily (pre-1987) or every 2-7 days (1987-89). This information, coupled with nest architecture measurements taken the following spring, allowed us to estimate which cell the bee was constructing on the day the nest was first located. Assuming that the bee takes approximately one day to complete a cell, we estimated the dates on which the nest was begun and finished. When the nest was completed, it was removed from the block, and replaced with an empty nest of the same bore size.

Each completed nest was stored in a large centrifuge tube with cloth covering the opening. Tubes were placed in wooden overwintering boxes built to fit the hutch shelves. Prior to 1987, completed nests were brought to Channing to overwinter, in order to avoid vandalism and marauding animals. However, starting in 1987, nests were left in overwintering boxes at the site where they were constructed. Starting in 1988, we took care to insure that nests were oriented in their original direction. Overwintering boxes were not left on hutch shelves as in the past, but rather were elevated about a foot off of the ground and camouflaged with branches, bark, and leaves in order to avoid vandalism. Fortunately overwintering boxes have not been vandalised at any of the sites, although hutches have been damaged and have disappeared during the winter.

Nest Architecture Measurements

Nests constructed by M. relativa during 1985 were measured after bee emergence, in November and December, 1986. Nests constructed during 1985 by M. inermis were measured after emergence in August, 1987. Most 1986 M. relativa nests were measured before emergence in 1987, so that we would know with certainty the species and sex of the occupant of each cell. The 1986 M. inermis began to emerge in spring 1987 before we began measuring their nests, so most M. inermis nests were measured after bee emergence. The 1987 and 1988 nests were measured sufficiently early in May of 1988 and 1989 that we were able to complete nest measurements of both species before they emerged in June and July.

After recording nest number and bore diameter, nests were split open lengthwise with a chisel. Total bore depth, non-reproductive spaces (basal space, vestibular spaces, associated caps, nest plugs, and indentation) were measured with the cells intact. Each cell was then removed and measured

from the base of the cell to the position of the outermost leaf in the cell cap (Fig. 5). Cell lengths measured after emergence are likely to be somewhat more variable than cell lengths measured before cell emergence, because emergence damages the cell cap. Thus it is sometimes difficult to determine where the edge of the cell cap starts.

The nest number that is written on each nest includes information on the site where the nest was created, so nest architecture measurements of pre-1988 nests were not blind. We doubt that knowledge of the nest site affected our measurements. However, in response to reviewer concern, we made blind measurements of the 1988 nests in May, 1989. Before nest measurements were made, students who do not measure nests spent a day crossing out the current nest numbers and replacing them with a random number independent of site. A data base not available to the nest measurers recorded the original nest number, and the random code number assigned to it. Nests were then measured without knowing at which site they were constructed. After all measurements were complete, and emergence was complete, the random number was associated with its original nest number including site.

Since more than one person measures nests, while changing nest numbers to code numbers, we attempt to divide the nests equally by site and date of nest initiation among all measurers. Thus individual biases in measurement are distributed evenly over sites and dates. In addition, in 1987 39 M. relativa cells were re-measured to determine within- and between-individual measurement error. Twenty cells were measured three times by each of the four individuals measuring nests. An additional 19 cells could only be measured 1 or 2 times by each measurer, because they were damaged by the multiple measurements.

Estimates of cell volumes were calculated using cell length and bore diameter measurements, assuming cylindrical cells.

Emerg nce Data

Nests created in 1985 were checked daily in the spring of 1986 for bees that had emerged from the nest and were in the tubes. For nests created in subsequent years, after measurement in the Spring, cells from which nothing had yet emerged were placed in individual plastic culture tubes or rearing dishes, and labeled with nest and cell identification numbers. In 1987 and 1988 tubes were kept in the Crystal Falls Laboratory at room temperature (approx. 68°F) until emergence. Because of concern about high 60hz EM fields, beginning in 1989 unopened nests and rearing tubes were kept at a holding site constructed by the ELF Small Mammal and Bird Project in woods south of Crystal Falls. Nests were brought to the Crystal Falls lab only briefly for measurement. There they spent up to 6 hours outside the house where 60

hz fields were low, and no more than 2 hours in the lab for measurements. In 1990 this protocol will be repeated. In addition, lab measurements will be made in a wire mesh "Faraday" cage constructed by IITRI to minimize exposure of developing bees to electric fields. After nest architecture measurements are complete, cells in tubes were returned to the holding site. Cells were checked daily for emergence. In all years, date of emergence, species, and sex of offspring were recorded.

Whenever possible, two or three bees from each 1986 - 1988 M. relativa nest were saved for dry weight measurements and for confirmation of species identification. In 1988 and 1989, M. inermis individuals from 1987 and 1988 nests were similarly saved for dry weight measurements. Dry weight is a more direct measure of parental investment per offspring than is cell length or volume. Some authors (eg., Cane, 1987) and one of our reviewers have suggested that measurement of hard body parts is a better indicator of body size than is weight, although the two are correlated (Cane, 1987). Field collected bees, which vary in age and foraging status, may be especially variable in weight. Since bees in this study were collected within hours of emergence without being released, their crops were empty. Thus much of the variability in weights that would be expected from a sample of field collected bees was eliminated. Measurement of hard body parts may still be a better measure of body size, but since we have weight data for our bees from a number of years, since it is relatively easy for us to make weight measurements, even in the field, and since we would have to remeasure many pinned specimens to get measurements of hard body parts, we prefer to continue taking weight measurements at this time.

Weights were obtained by drying bees in a desiccator over P_2O_5 to constant weight. Constant weight was defined as two weights taken 48 hours apart that were within 0.5mg of each other. The lower of these weights was used in analyses.

Bees were identified by G. Dahlem, V. Scott, and K. Strickler based on Mitchell (1962), and by comparison with reference specimens provided by T. Griswold, ARS Bee Laboratory, Utah State University, Logan Utah.

The remaining adult bees were released at the sites where their nest had been constructed the previous summer. The Faraday cages mentioned above are intended to insure that released bees were not affected by 60 hz electric fields when nest architecture measurements were taken. Effects of 60 hz fields might be mistaken for (or might mask) effects of the ELF antenna's 76 hz fields, and affected bees might alter the genetic makeup of natural populations. Parasites were collected and not released.

Cells that showed no signs of emergence were opened in August (1986-88 nests), or when the nest was measured (1985 nests). Contents were recorded to indicate at what stage mortality had occurred.

Leaf Counts

The number of elongate leaves that were used to construct a cell was determined for 1985 M. inermis cells and 1986-1988 M. inermis and M. relativa cells that were still in good condition once emergence was complete. Leaves lining M. inermis cells overlapped, but were easy to tease apart and count. Leaves lining M. relativa cells were smaller, and were "glued" together so that a microscope was often needed to determine where one leaf ended and the other began. When in doubt, leaf counts for M. relativa cells were not recorded.

Data Entry for Nest Architecture, Emergence, and Leaf Count Data

Nest architecture measurements, emergence records, and leaf counts are recorded manually in the spring and summer on data sheets for each nest. In the fall, these data are typed into an R-Base file on a Zenith personal microcomputer, where an initial check for errors is made. The R-Base file is then down-loaded to the VAX 11/730 computer (VAX/VMS operating system) in the Department of Entomology at MSU. Here they are checked further for errors, and loaded into several files in INGRES, a relational data base management program on the VAX. Finally, relevant subsets of the data are transferred from INGRES to SAS data files for statistical analysis.

Nest Activity

One or more observers have gathered data on behavior of individual bees at the nest every year since 1983. In the 1986 Annual Report, we decided to focus on the collection of round pieces of leaf (LO trips) used in capping a cell. Analysis (1986 Annual Report, p. 20-21) suggested that this was the most consistent of the three main behaviors in nest construction (collection of pollen, collection of elongate leaves for cell lining, and collection of round leaves for cell caps). LO trips probably involve fewer extraneous behaviors such as sunning or taking nectar than do pollen or elongate leaf collecting trips. Thus residuals for the duration of LO trips could be normalized for statistical analysis. Consistency in LO trip durations probably results from the necessity to cap the cell rapidly to avoid parasitism after laying an egg.

Prior to 1987 each observer watched a single bee for several days in succession, until the nest was complete. This protocol generated a great deal of information on the variability in behavior within a bee, but less information on between-bee variability. In 1987 - 1989 field seasons we maximized the number of bees timed per day, rather than timing one bee for

long periods of time. Observers became adept at locating a bee that was about to lay her egg, and were able to focus on timing the first few LO trips that the bee made after laying her egg. Generally, we tried to time 5 such trips in succession before searching for another bee that was about to collect LO leaves. Occasionally the bee would complete a cap in fewer than 5 timings. The observer sometimes would time more than 5 LO trips if no other bees were active. Number of trips timed for a bee on a given day ranged between 1 and 18. Occasionally the observer missed recording the time of the first few trips. In 1987 we did not try to record the number of LO trips that the bee made before we began timing. Our 1987 analysis suggested that this "trip rank" number is important (1987 Annual Report), because LO trips increase in duration with each successive trip. Thus, during the 1988-1989 field seasons we attempted to record this number when timings were made. Only LO durations for which this trip rank order was known are used in the current analysis.

During the 1987 - 1989 field seasons, four observers were rotated between sites every 3 to 4 days, so that biases between observers would be distributed evenly between sites and dates. On a given day, two observers visited a control site and two an experimental site.

Prior to 1987, the duration of LO trips was determined by using a watch to record the hour, minute, and second that the bee left the nest and returned to the nest. Since 1987, we have used portable Tandy 102 computers that are programmed as event recorders. When the program was activated, the observer was prompted for information on the nest number and site, and some weather data (see below). The program automatically numbered the observed activities in sequence. Hitting the space bar recorded the time to the nearest second at which the bee left the nest or returned to the nest. A single letter code was used to indicate what cargo (e.g., LOs), if any, the bee brought back to her nest. These data were down-loaded to a Zenith personal computer at our field headquarters, and later transferred to an INGRES data base file on the VAX computer in the Department of Entomology. Durations of each trip were calculated in INGRES by subtracting the time when the bee left the nest from the time when the bee returned.

Because behavior of insects is often affected by such environmental factors as temperature and wind speed, foraging trip durations could be correlated with weather conditions. Prior to 1987, air temperature, relative humidity, solar radiation, rainfall, barometric pressure, wind direction, and wind speed were monitored automatically with Model TI-5X instrumentation modules at one experimental (F1) and one control (CL) site. The instrumentation did not always function properly. In 1987 we did not have time to set up the automatic weather equipment until the beginning of August. Then we principally wanted to determine what equipment was functional and what was not. Soon after setting it up, one of our batteries was

stolen. We have not had time to evaluate the availability of weather data from these automatic systems, or to attempt appropriate correlations. After 1987 we did not take the time to set up the automatic weather instruments.

Some weather data were recorded in the event recorders as each bee was timed during the 1987-1989 field seasons. This included sun conditions (sunny, partly cloudy, cloudy, rain), temperature in the shade on the same shelf as the bee's nest, shading of the block in which the bee's nest was found, relative humidity calculated with a sling psychrometer, average wind speed and speed of wind gusts measured with a Dwyer Portable Wind Meter (hand held). Although our measurements of solar radiation, relative humidity, and wind speed may be crude, they are better than nothing (as we had in the past for C5 and F2), and may give a better indication of conditions around the nest than did the automatic monitoring equipment, which was not always near the appropriate hutch. This weather data is recorded on Tandy computers, down-loaded to the Zenith and then to an INGRES file on the VAX computer (as described above for LO durations).

Description of sites

Three sites are located on Copper County State Forest Property in Dickinson Co. in the Upper Peninsula of Michigan. A fourth site (C5) is located in Iron Co. on property leased by the Michigan Department of Natural Resources to Champion Paper Company. Permission to use these sites is gratefully acknowledged.

The C5 site is located 6.7 km south of Route 69 and about 0.8 km west of Camp 5 road in Iron County, Michigan (Township 42N, Range 31W, Section 13). The area has recently been logged, and nearby forests continue to be logged within a km. of our hutches. An abandoned railroad bed runs N-S just west of the hutches. Camp 5 creek runs through the site, creating a cut-over swamp and flood plain. Four hutches are located at the edge of this flood plain, and two hutches are located in an open depression next to the abandoned railroad bed. Nearby woods consist primarily of Populus tremuloides, with occasional Larix decidua, Picea glauca, Pinus resinosa, and Prunus serotina. Shrubs in the vicinity include Alnus rugosa, Vaccinium sp., Salix sp., Spirea alba, and Rubus allegheniensis. Herbaceous plants include Cirsium palustre, Fragaria virginiana, Hieracium spp., Trifolium spp., and Solidago spp.

The CL site is located about 1.7 km north of Route 69 on the east side of County Line Road, in Dickinson Co., (Township 43N, Range 30W, Section 19). Logging continues within a km or so of the hutches. This site has very sandy soil and is the driest of our sites. Hutches are located at the edge of clearings in Populus tremuloides woods, with occasional Acer saccharum, Betula papyrifera, Abies balsamea, and Pinus resinosa. Two hutches are adjacent to a

patch of trees north of a logging road through the sandy clearing. Two are east, and two west of a marshy, low lying area south of the logging road. Hieracium aurantiacum carpets the ground at this site in June, if rain has been sufficient. Bracken fern is common near the east hutches which are in a shadier location than the others. Other flowering plants that are common in the area include Cornus canadensis, Campanula rotundifolia, Fragaria virginiana, Epilobium angustifolium, Rubus spp., Solidago spp., Vaccinium spp., and Prunus pensylvanica. Small patches of Cirsium palustre grow in the marshy area south of the logging road.

Low numbers of M. inermis nests at the CL site, especially in 1986, prompted us to transplant about 90 Cirsium spp. plants (a common pollen source at other sites) to the CL site in April, 1987 and 50 plants in June 1989 to try to increase the numbers of M. inermis that nested there.

The F1 site is located south of Turner Road, and north of the Ford river, 20 km east of Channing. (Township 43N, Range 29W, Section 14). The hutches are located at the edge of a flood plain, bordered on the north by a Red Pine plantation, and the south by vegetation along the river consisting of Populus balsamifera, Populus tremuloides, Fraxinus nigra, and Alnus rugosa. The pine plantation has been clear cut to allow for the construction of the ELF antenna, which runs NE-SW through the site. Two hutches are east of the antenna, at the north edge of the flood plain. Two are a similar distance west of the antenna. Two are in a shady clearing further west of the antenna at the northwest edge of the flood plain. Flowering plants near the hutches include several species of Cirsium, especially C. palustre and C. arvense, Urtica dioica, Solidago spp., Hieracium aurantiacum, Hypericium perforatum, Aster spp., Rubus spp., Humulus lupulus, Linaria vulgaris, and Vaccinium spp.

The F2 site is located about 0.8 km south of the Ford River and the F1 site, along the clear cut for the ELF antenna. The soil is sandy. Three of the hutches are located on top of a hill at the edge of the clear cut west of the antenna, and along an old logging/hunting trail running west from the antenna. Three hutches are located in a valley east of the antenna. Nearby woods consist of Populus tremuloides, with occasional Picea glauca, and Pinus resinosus. Centaurea maculosa has increased since 1983 until it is now the most abundant flowering plant on the hill. Also abundant are Cirsium palustre, Fragaria virginiana, Hieracium aurantiacum, Coronilla varia, Prunus virginiana, Rubus idaeus, Solidago spp., and Trifolium spp.

ELF Antenna Operations

In interpreting results of this project it is important to know the general pattern of antennal operations over the past few years (Table 2). The Michigan Transmission Facility (MTF) began testing at 10% power (15 amperes) periodically from March - October, 1986 and with increasing

regularity from May - November, 1987 and January - July, 1988. Because analyses for 1986 and 1987 data did not show any effect of experimental and control areas on nest architecture and activity, we regard these years as non-treatment years. Starting July 6, 1988 and lasting until May, 1989, testing continued at 50% power (75 amperes). Nest activity data from 1988, analyzed in last year's annual report, also were not affected by experimental and control areas, so 1988 was regarded as a pre-treatment year for activity data. In this report, architecture data and mortality data from 1988 nests are analyzed (Table 2). Bees that constructed nests, and their overwintering offspring, were exposed to higher ELF EM fields than in previous years, but not yet to full power (150 amperes). Thus, if experimental and control areas still do not affect nest architecture or overwintering results, this does not preclude the possibility that full EM fields will have an effect.

Nests constructed in 1989 were the first to be exposed to standard 150 ampere operations. Readers should keep in mind that in this report, only nest activity data were collected while the MTF was operating at full power (Table 2). Nest architecture and mortality data collected under full power conditions will be analyzed in next year's annual report.

For detailed information on EM measurements at the sites involved in this study, see the technical reports, *ELF Communications System Ecological Monitoring Program: Electromagnetic Field Measurements and Engineering Support* prepared by IITRI for each year of the program.

Statistical Methods

The General Linear Models (GLM) procedure on SAS (Version 5) was used to analyze sources of variability in cell lengths and cell volumes (both species), leaves per cell (M. inermis) and LO trip durations (M. inermis). In these models, each cell or LO duration is treated as an independent measurement. As pointed out by one reviewer, this analysis may be inappropriate if cell lengths or leaves per cell within a nest, and/or LO durations within a cell capping bout, are autocorrelated. An alternative analysis would involve GLMs on the mean cell length or leaf number for a nest, or mean LO duration for a cell capping bout. However, taking a mean for each nest or capping bout does not account for systematic changes with cell order within the nest or with trip rank in a capping bout. Another possibility would be to restrict our analyses to the first cell in a nest, or the first LO trip duration in a capping bout. Alternatively, a multivariate GLM, testing lengths of cells 1-3, or LO trip rank 1-8 could incorporate more of our data in the analysis, while eliminating the problem of autocorrelation. We will continue to consider other analyses over the next year to account for the possibility of autocorrelation in our data.

In our current analyses, experimental vs. control areas (Exp) was treated as a random class variable. Sites nested in experimental and control areas (Sites[exp]), observers or measurers nested in year (doneby[yr]), and sex of offspring were treated as fixed class variables. Complete vs. incomplete nests were a random class variable in the analysis of cell length and cell volume. Cell order, number of cells per nest, nest diameter, bore depth and date of nest initiation were covariates in the analysis of cell lengths, cell volumes, and leaves per cell. Leaves per cell was a covariate in the analysis of cell length and cell volume. Rank order of the trip (trip number), time of day, and date of the trip were covariates in the analysis of LO trip durations. Time was also tested as a second order covariate in this analysis. Significance of time would indicate that LO durations are faster (or slower) during the middle of the day, as might be the case if LO durations are correlated with temperature. All other variables were fixed in the analysis. Type IV mean squares were calculated in all GLM analyses. This model is invariant to the ordering of effects in the model.

The ms of Site[exp] was used as the error term for testing the significance of Exp, and interactions between Exp and other variables in the models. This insures that differences between experimental and control areas are greater than any differences between the sites that comprise the areas. The ms of measurer[yr] was the error term for testing year, and interactions between year and other variables in the model. This insures that differences between year are greater than the differences between the measurers who took data in any given year. For M. inermis, the interaction between year and bore depth was included in most models because depths of the largest diameter bores varied between years.

Our models tested for significance of the interaction between year and Exp, using the ms of Site[exp] as the error term. If significant, this interaction term indicates that one area has shown a greater change between years than the other. Ideally this interaction term will not be significant before the antenna is operational. If the Exp main effect is significant but not the Exp*Year interaction, then we know that there are intrinsic differences between experimental and control areas that have nothing to do with the antenna. If the year main effect is significant but not the Exp*Year interaction, then we know that there are differences between years that have affected both experimental and control areas equally, as would be the case for climatic changes between years. If the Exp*Year interaction is not significant before the antenna is operational, but it becomes significant after the antenna is operational, the antenna is a likely cause of the difference. If the interaction term is significant before the antenna is operational, then the problem of detecting differences between experimental and control areas will be much more complex.

Factors such as nest diameter, date of nest initiation, and offspring's sex are included in the model because if they contribute to variance in cell lengths and/or volumes now, then changes in these factors due to ELF EM fields are possible. Such changes will be the underlying cause of differences between experimental and control areas due to ELF EM fields, if such differences are found. For example, if sex of offspring contributes significantly to the variance in cell lengths before the antenna is operational, then cell lengths could decrease after the antenna is operational because a higher proportion of male offspring are produced.

A Shapiro-Wilk statistic for N<51 and a Kolmogorov D statistic for N>=51 in the Univariate procedure of SAS were used to test for normality of residuals in models of LO trip durations, cell lengths, cell volumes and leaf counts. The significance level used in these tests was 0.05. Log or loglog transformations of the data were sometimes required to meet the assumption of normality. When used, such transformations are discussed in the Results section.

Minimum detectible differences between experimental and control areas [Exp] were tested with a modification of Cochran and Cox's (1975) formula (Zar, 1984 p.135). Conservative sample size estimates were based on numbers actually collected between 1985 and 1987 for the two control sites combined or the two experimental sites combined, whichever was smallest. The value of population variance s^2, used in calculating minimum detectable differences, was the site[exp] mean square because this mean square value is used as the error term for testing Exp and Exp*Year (Zar, 1984 p.260). Values of α and the power of the test (1-β) were 0.05 and 0.9 unless otherwise stated. We would prefer to test for the minimum detectible difference for the Exp*Year interaction, but we do not know how such a test would be made.

A two-way classification mixed model ANOVA was used to analyze within- and between- measurer components of cell length variability (Sokal and Rohlf, 1969, p. 313). In this analysis, measurer was a fixed-effect, whereas cells measured was a random effect. The interaction between measurer and cells was also included in the initial run of the model. The error mean squares gives within-measurer variability.

The Categorical Data Modeling (CATMOD) procedure on SAS was used to compare distributions of cells per nest from experimental and control areas. This statistical program fits linear models to functions of response frequencies for discrete data; ie., it is an extension of the GLM procedure for continuous data that was used in the analyses of cell lengths and volumes. The program uses a Wald statistic (which approximates a chi-square distribution for large sample sizes) to test hypotheses about linear combinations of the parameters in the model. As with the GLM tests previously described, we tested for significance of experimental vs. control

areas (Exp), sites nested in Exp areas (Site [exp]), years, and the interaction between Exp and years (Exp*Year). The level of significance of all tests was $\alpha =$ 0.05.

Proportion of nests oriented in a N-S vs. E-W direction was tested against the prediction of equal proportions in each direction. When at least 10 nests were constructed at each matched set of hutches for a given year and site, a heterogeniety Chi-Square Goodness of Fit test was used (Zar 1984, p. 49). When a preference was discerned in the nest directions accepted by bees at a hutch set or site (ie., the null hypothesis in the Goodness of Fit test was rejected), then a log-likelihood ratio heterogeniety contingency table analysis (Zar 1984, p. 67-68) was used to determine if the preference was the same for all years at a given hutch set, and for all hutch sets at a given site.

Proportion of mortality in the overwintering prepupal stage was tested with the ANOVA procedure in SAS in a randomized block design. Proportions were transformed using a Freeman and Tukey arcsine transformation (Zar 1984, p. 240):

$$\text{pft} = 1/2 \, [\text{arcsine } X/(n+1) + \text{arcsine } (X+1)/(n+1)].$$

In the 1988 Annual Report, two additional transformations were tried in ANOVAS. These included Ascomb's arcsine transformation (Zar 1984, p. 240), and the Probit of Rao's transformation (Rao 1965). Since all three transformations gave very similar results, we have decided to stick with Freeman and Tukey's arcsine transformation, which is preferable for small proportions (Zar, 1984, p. 240-241). Analysis using this transformation had the greatest r^2.

Resulting values were analyzed in an ANOVA to determine whether Site[exp], year, Exp, Exp*Year, contribute significantly to variability in proportions. The ms of Site[exp] was used as the error term for testing the significance of Exp, and interactions between Exp and other variables in the ANOVA. Calculation of proportion of prepupal mortality was complex, and will be explained in the results section.

Minimum detectible differences for ANOVAs of proportion data can be determined using a jackknife technique. We have not had time to complete the simulations for this year's data as of this writing. However, the technique was reported in the 1988 annual report for prepupal mortality. In brief, the average proportion mortality for 1985-87 was calculated for each site. This average was assigned to hypothetical data for a fourth year, except that the proportion mortality for the experimental sites was increased stochastically by an average of a specified amount (2 fold, 2.5 fold, 3 fold). A new variable, called ELF was set to 0 for 1985-87, but set to 1 for experimental sites in the fourth year, simulating the presence of ELF EM fields. 100 realizations of the

modified data were made for each average increase in proportion of prepupal mortality. We looked for significance (α = .05) of the ELF variable in at least 75 of the 100 realizations for an acceptable minimum detectable difference.

IV. NEST ARCHITECTURE RESULTS

Bee Abundance

Table 3 summarizes the number of nests of the two species for which we have data on cell lengths, and an estimate of the number of complete nests created in 1989. Some 1985 M. inermis nests were not included in our measurements because they were used by Dr. Fischer in experiments on diapause. The 1983 nest architecture data have not yet been incorporated into our analysis. They are, however, being edited to make them comparable to the 1985-1986 data. Nests constructed in 1989 will be measured in the spring of 1990.

Cumulative numbers of nests constructed each week at the four sites are presented by year in Figure 6. Final nest numbers are underestimates for M. inermis in 1985 and for both species in 1989, as explained above. There are differences between sites·and years in dates of first and last nest construction, and in rates of nest construction through the season. Generally, nesting started early in 1987; late in 1985 and 1989. The rate of nest production for M. inermis tended to be greatest in mid to late July, while M. relativa nesting activity tended to be most rapid earlier, or to be fairly constant over the season.

Of the 90 Cirsium transplanted to the CL site in 1987 in an attempt to increase the M. inermis population, about one fourth of them survived and bloomed. An additional 50 plants were transplanted in June, 1989 into the marshy area of CL. We do not know how many·successfully bloomed, because numerous endemic thistle bloomed in the marsh in 1989. These transplants probably did not provide enough additional resource to affect bee populations. We hope that our efforts are not entirely in vain, since thistle seeds produced by the transplants may increase the Cirsium population in future years.

No new transplants were made in 1988. In 1988, transplants would not have survived the spring drought. Unfortunately, the drought prevented many spring flowers, particularly Hieracium aurantiacum, from blooming. This had a devastating effect on bee populations at the CL site, especially M. inermis, which depend heavily on hawkweed in June. In 1989, nesting began two to three weeks late due to a cool spring, by which time hawkweed was already past peak bloom. This differed from 1987, when hawkweed bloom and bee nesting seemed to be synchronized. Flower abundances recovered in 1989, so once the bees began foraging, their nesting activity reached typical or even record levels at most sites (eg., M. inermis, F2, Table 3, Fig. 6).

Hypothesis 1: The average size (length and volume) of cells for each offspring, and/or the average number of cells produced per nest is unchanged by exposure to ELF electromagnetic fields.

M. relativa

The GLM procedure for analysis of cell lengths eliminates from the analysis records that are incomplete for any of the variables in the model. A large number of cell records did not include number of leaves per cell, and sex of offspring for this species. Leaves per cell were not counted in 1985, and often could not be counted in subsequent years when cells were mouldy or when leaves could not be separated. Sex of offspring was missing for all cells where mortality occurred before the pupal stage, or where a parasite emerged, and for cells where emergence took place before the cell was measured (most 1985 cells). Date of nest initiation, which in previous Annual Reports was missing from 1986 F1 nests, was included in all analyses this year. (A missing notebook that contained this data was found in spring, 1989). The GLM test was repeated several times with different subsets of the data so that we could test whether all of these variables contribute to variance in cell length. First, we tested cell lengths with the maximum number of cells (Table 4), by not including number of leaves per nest, or sex of offspring, in the model. The Exp mean squares from this GLM was used to estimate minimum detectable differences between control and experimental sites (see below). Second, we tested only cells from nests with diameters between 5.0 and 7.0 mm (bore size 4) (Table 5), since these are most comparable with nests from 1987. Third, number of leaves per cell were included in the GLM test (Table 6). The fourth GLM test included only cells for which the sex of the M. relativa offspring was certain (Table 7). This year for the first time, we also include separate analyses on cells with male offspring and cells with female offspring (Tables 8 & 9). Residuals were not significantly different from normal for all of these analyses.

The analyses in Tables 6 & 7 were repeated using only nests with diameters between 5.0 and 7.0 mm. with similar results. Full GLM tables are not presented here, to spare the reader sorting through more of the same type of table. Instead, all analyses are summarized in Table 10 and compared with the results reported in the 1988 Annual Report. Overall patterns of significant and non-significant variables are apparent in Table 10 and are discussed below.

Mean cell length was 11.1mm for M. relativa in nests with diameters between 5.0 and 7.0mm. The models accounted for only 13-31% of the variance in cell lengths (see r^2 in Tables 4-10). This is an improvement over the analyses in the 1988 Annual Report. Adding sex of offspring substantially increased the proportion of the variance accounted for in the models. The

model explained the highest proportion of the variance when only cells with female offspring were included in the analysis (Table 9). Low r^2s in these analyses may be related to the low overall variance in cell lengths, only 7-9% of the means (see CV in Tables 4-10).

In all of the GLM tests, Exp and Exp*Year did not contribute significantly to variance in cell lengths. This suggests that testing of the ELF antenna at half power (75amps) during the summer of 1988 did not have any effect on the length of M. relativa cells created during that year. Furthermore, there are no intrinsic differences between experimental and control areas contributing to variability cell lengths.

Differences between measurers contributed significantly to variance in cell lengths in all GLM tests (Table 4-10). In particular, 1989 cells measured by KS were larger on average than were cells measured by BZ or VS (Table 11). Since the same three measurers will have this task in 1990, we plan to examine our methodology in more detail to try to improve standardization and to decrease the contribution of measurer on variability, if possible. We have, however, taken pains in our blind measurement technique, to assure that measurer biases are distributed evenly over all sites. (See below for more discussion).

Cell lengths consistently decreased with cell order from nest base to cell entrance (Tables 4-10, 12). Since female offspring tend to be found in inner cells and males in outer cells, we wondered if cell order contributes to variability in cell length because it is correlated with sex, or if this pattern also exists within a sex. As indicated in Tables 8 & 9, cell order contributes significantly to cell length variability for both cells with male and cells with female offspring. Thus the significance of cell order in previous tests is not due exclusively to offspring sex. Date of nest initiation, cells per nest, measurer (both sexes) and nest diameter (male cells only) are also significant contributors to variability in cell length when sexes are separated in the analysis.

Cell length decreased slightly but significantly with date of nest initiation in all tests (Tables 4-10). Cell length decreased slightly but significantly with nest diameter in all tests (Tables 4-9) except female cells only (which are most abundant in the largest diameter bores). Differences between years were not significant in these analyses, although year differences have occasionally been significant in past analyses (Table 10). Number of leaves per cell contributed significantly to variance in cell lengths, although it was a minor contributor compared with other variables (Table 6).

As in the past, offspring sex contributed significantly to cell lengths (Tables 7,10). Cells with male offspring were significantly smaller on average (length=10.9mm N=1213), than cells with female offspring (11.8mm N=246).

When sex is included in the model, the proportion of variance in cell lengths explained by the model is approximately doubled in comparison to tests without offspring sex in the model, as was noted in previous annual reports.

GLMs of cell volume were not recalculated this year in order to allow extra time to refine the analysis of overwintering mortality, and to analyze nest orientation. The results of cell volume analyses from the 1986 Annual Report are summarized in Table 13. They are very similar to the results for cell length, except that the correlation between cell volume and diameter is much stronger than the correlation between cell length and diameter, and thus the proportion of variability explained by the model (r^2) is larger for cell volume than for cell length.

In the 1987 Annual Report, we expressed concern that bore diameters of 5.5 mm might bias the sex ratio towards males, since most females in 1986 nests were in larger diameters. Therefore, we added some 6.0mm nests in 1988. Our fears proved to be unfounded (Table 14) since 1987 nests had sex ratios as low as or lower than in other years. In 1985 4.8 males were produced for every female. In 1986, a drought year with low flower bloom, 5.4 males per female were produced. In 1987, a wet year with high flower bloom, 3.1 males per female were produced. Since 1988 was a drought year with poor flower bloom, we predicted a high sex ratio for nests produced in 1988, in spite of the inclusion of some 6.0mm bore diameters. The 1988 sex ratio was 4.5, higher than 1987, suggesting that flower resource availability is more important than nest diameter in affecting sex ratios.

Within and Between Measurer Variability. As mentioned earlier, differences between measurers consistently contributed to the variance in cell lengths in all tests performed (Tables 4-10). Mean cell lengths for individual measurers varied from 10.59mm (ND, 1985) to 11.33mm (KS, 1987) (Table 11). The range of means between measurers was greatest for 1986 cells, when four measurers were involved (11.11-10.65=0.46mm). It decreased considerably for 1987 cells (11.33-11.05=0.28mm). Although we expected the differences between measurers to remain low, since at least two of the same measurers (KS and VS) were involved with both 1987 and 1988 nests, this was not the case. Rather, the range of means for 1988 was 0.43, almost as great as in 1986.

In order to better understand the contribution of measurer differences to cell length variability, in 1987 39 M. relativa cells were measured up to three times by each measurer after the cell was originally measured. In an initial two-way mixed-model ANOVA there was no significant interaction between measurers and cell measured. This indicates that although the mean cell length differed between measurers, the magnitude of the differences between cells was the same for all measurers.

The interaction and error variances were pooled by rerunning the ANOVA without including the interaction term in the model. This omission had the additional advantage that the residuals from the model were normally distributed, whereas with the interaction term the residuals were not normally distributed. Each person measured each cell an average of 2.55 times; this value was used to compute the relative contribution of within- and between- measurer variance to the total variance (Table 15). 75% of the variance was between cells, while only 25% was between and within measurers. Variance within measurers (15%) accounted for more of the measurer variance than did variance between measurers (10%). In this analysis the mean cell length was 10.5mm, and the overall coefficient of variation was only 3.6%, or about 0.4mm. Thus, measurer variance accounts for only about 0.1mm. In the full analysis, the overall mean cell length was 11.0mm with a CV of 8.9%, or about 1.0mm. Thus, our analysis suggests that measurer variance accounts for about 0.25mm of the total variance.

Because the contribution of the within-measurer component of variance is greater than the between-measurer contribution, and the sum of both components accounts for a relatively small percent of the variance and a very small absolute amount of variation, we concluded in the 1988 Annual Report that further steps to reduce this variance are unnecessary. However, in light of the increase in discrepancy between measurers of 1988 nests, steps will be taken in 1990 to decrease these differences, as mentioned above.

Minimum Detectible Difference Between Experimental and Control Areas. Assuming a minimum of 109 cells per site per year (the smallest sample size we have seen so far, F1, 1987), we expect a minimum of 436 cells over 4 years. Using n=436 and $s^2 = 21.18$ (Table 4, SS for Exp), we calculate that we should be able to detect at least a 1.0mm difference (9% of the mean) in cell lengths between control and experimental areas with a power of 90% and $\alpha = 0.05$. These projections are an improvement over projections in last year's annual report. Thus, in order to detect an effect of ELF EM fields on cell length, experimental and control areas will have to differ by slightly more than the difference caused by offspring sex. It will take more than a change in sex ratio in order to detect differences between experimental and control areas.

M. inermis

GLM tests on cell length variance were applied to four different subsets of the M. inermis data. The first GLM model (Table 16) includes only cells from bore size 7 (diameters > 9.5mm) nests. The Exp mean squares from this GLM was used to estimate minimum detectable differences between control and experimental sites (see below). The second analysis includes all nest diameters, but only cells for which sex of offspring was known (Table 17). Table 18 includes only cells with male offspring, while Table 19 includes only

cells with female offspring. In analyses including offspring sex, sample sizes for 1985 and 1986 are much reduced because only a small number of offspring could be associated with a specific cell during those years. Number of leaves per cell was included in all models because values were determined for all years.

In the first two models, residuals were significantly different from normal (P<0.01, Kolmogorov test), apparently because of a few small cells lengths, rather than any general bias in the overall distribution (Fig. 7). We believe that the qualitative results of the GLM analysis are sufficiently robust to be worth reporting. When cells with male and female offspring are analyzed separately, the residuals are not significantly different from normal (P>0.15).

Mean cell length was 15.6 mm for M. inermis cells with male offspring, and 16.8 mm for cells with female offspring. As with M. relativa, variance in both factors was low, only 6-7%. The models of cell length account for 42-52% of the variance. This is an improvement over the GLMs for cell length reported last year.

Neither Exp, Year, nor Exp*Year contributed significantly to variance in M. inermis cell lengths. This suggests that testing of the ELF antenna at half power (75amps) during the summer of 1988 did not have any effect on the length of M. relativa cells created during that year. Furthermore, there are no intrinsic differences between experimental and control areas contributing to varability cell lengths. As with M. relativa, differences between measurers (measurer [yr]) made a significant contribution to cell lengths. Cells measured by KS were larger than cells measured by other measurers (Table 20). As mentioned earlier, an evaluation of methodology to improve standardization and to decrease the contribution of measurer on variability will be undertaken in the spring when 1989 nests are measured.

Although cell lengths decreased significantly with cell order from base to nest entrance when all nests greater than 9.5mm in diameter were included in the analysis (Table 16), cell order did not contribute significantly to cell length when sex of offspring is included in the model, or when male and female offspring were analyzed separately (Tables 17-19). Thus, decreasing cell size in the nest can be accounted for by the pattern of inner female cells and outer male cells (Table 12).

Aside from measurer, cells per nest was the only variable that contributes significantly to variability in female cell lengths, and these two variables account for over half of the variability (r^2=0.52, Table 19). However, only about 250 cells were known to contain females, compared with close to 1100 cells known to contain males. For male cells, few variables did not

contribute significantly to cell length variability. Non-significant variables included Exp, Year, and Exp*Year, and date of nest initiation (Table 18).

Sex ratios have generally decreased over the years of the study (Table 21). During 1985 and 1986, high sex ratios may have been an artifact of short bore depths (Stephen and Osgood, 1965). Sex ratios were generally higher in the drought year of 1988 than in 1987, perhaps due to reduced resource levels (Torchio and Tepedino, 1980).

Minimum Detectible Difference Between Experimental and Control Areas. Assuming a minimum of 31 cells per experimental or control area per year (the smallest sample size we have seen so far, control areas in 1986), we expect a minimum of 124 cells over 4 years. Using n=124 and $s^2 = 27.35$ (Table 16, SS for Exp), we calculate that we should be able to detect at least a 2.16mm difference (14% of the mean) in cell lengths between control and experimental areas with a power of 90% and $\alpha = 0.05$. These projections are an improvement over projections in last year's annual report.

Thus, in order to detect an effect of ELF EM fields on cell length, experimental and control areas will have to differ by about twice the difference caused by offspring sex. It will take more than a change in sex ratio in order to detect differences between experimental and control sites.

Offspring Weights. In the 1987 Annual Report we questioned the necessity to analyze the variance in cell volumes, because volumes are highly correlated with nest diameters. We suggested that the answer to this question depended on whether offspring weights correlate best with cell length or with cell volume. Dry weights of some M. relativa offspring from 1986 nests were measured in hopes of addressing this question. Furthermore, both live and dry weights of a sample of both species from 1987 and 1988 nests were measured.

Tables 22 and 23 present mean dry and live weights by sex, year and site for M. relativa and M. inermis, respectively. No statistics have been accomplished with these data. However the means for M. relativa suggest that there are differences between years for both sexes, with weights reduced during the drought years of 1986 and especially 1988, when hawkweed did not bloom. There may also be differences between sites (eg., bees from CL tend to be smaller than bees from other sites). There do not appear to be systematic differences bewteen experimental and control areas. We have not yet determined whether there is any correlation between offspring weight, and cell lengths or volumes.

Weights were not taken for some control sites in some years for M. inermis because of low sample sizes. Weights seem to be somewhat lower in 1988 than in previous years for M. inermis, as was the case for M. relativa.

After weighing, the bees were pinned and identified. All of the small Megachile bees from 1986 and 1987 nests were confirmed as M. relativa. The 1988 bees are currently being pinned.

Number of cells per nest

Number of cells per nest ranged from 1 to 12 for M. relativa (Fig. 8a,b). Mean number of cells per nest for each site and year are given in Table 24. In a CATMOD analysis fo cells per nest we used four categories to insure that frequencies were greater than five per category for all sites and eyars. The categories were: nests with 1 or 2 cells, nests with 3 or 4 cells, nests with 5 or 6 cells, and nests with seven or more cells.

There were significant differences in the distribution of number of cells per nest between sites, Years, Exp, and the interaction between Exp and Year (Table 25). If the Exp differences are real, they occur before the ELF antenna became operational. The Exp*Year interaction is presumably significant because 1987 is the only year in which experimental nests had fewer cells than control nests (Table 24, bottom). Thus, the Exp and Exp*Year differences are not related to ELF EM fields.

Furthermore, in the CATMOD analysis, unlike GLM analyses, Exp and Exp*Year are not compared with Site[Exp] differences. Examination of the means by site (Table 24, top) suggests that the differences between the two control sites (e.g., 6.17 vs. 4.05 in 1987), or between the two experimental sites (e.g., 6.46 vs. 3.72 in 1985) are in fact greater than the differences between the overall mean of control sites vs. experimental sites (e.g., 3.85 vs. 5.10 in 1986). Thus, the Exp and Exp*Year should probably not be considered significant.

Differences between years do appear to be real, and are probably related to resource levels, since the number of cells per nest was lower during drought years (1986, 1988) than during other years. These results support our speculation that decreased offspring weight in 1986 and 1988 are related to reduced resource level in drought years. The generally lower number of cells per nest at F2 is probably due to the presence of an aggressive nest competitor, Eumegachile pugnata, in high numbers at this site. We are not sure why cells per nest was high at F2 in 1986.

Because of high variability, and little consistency between sites over the years in cells per M. relativa nest, we expect that it will be difficult to recognize effects of the ELF antenna on cells per nest, should any occur in future years. Intrinsic differences between sites within experimental and control areas can be as great as two cells per nest, so a change of three or more cells per nest will need to occur between experimental and control areas to be biologically significant. We propose to continue analyzing these data, in case

major changes do occur, because data on number of cells per nest takes no extra effort to collect while we are making nest architecture measurements. Drastic reductions in hon..ybee reproductive output on exposure to high voltage transmission lines (see Introduction) suggest that majo: changes might also occur in Megachile nests, although the electric shock from induced honeybee hive currents that are responsible for this result (Bindokas et al., 1988) are not likely to occur in Megachile trap nests.

Number of cells per nest ranged from 1 to 7 for M. inermis (Fig. 9). The deeper the nest, the more cells can be constructed. Therefore, in analyzing cells per nest for M. inermis, we compare only 1987 and 1988 nests, when bore depth was routinely 140mm and only bore size 7 nests (diameter > 9.5mm) were provided. Mean number of cells per nest for each site and year are given in Table 26. The experimental sites have more cells per nest than do control sites, as confirmed by CATMOD analysis (Table 27) using two categories (1-4 cells or 5-7 cells). Because there were no significant differences between sites within experimental or control areas, the Exp differences are real for this species. No significant Exp*Year interaction indicates no effect of ELF EM fields at 50% power. It should be easier to detect ELF related changes in cells per nest for M. inermis in future years, than it will be to detect such changes in M. relativa nests.

There were no significant differences between years for M. inermis. Unlike M. relativa, the larger M. inermis did not produce significantly fewer cells per nest in the drought year of 1988. This is surprising, since one might expect lack of resources to affect a large species more than a small species. Apparently these two species responded differently to the drought: The small species produced more nests in 1988 than in previous years (Table 3), but with fewer cells per nest (Table 24) and smaller offspring (Table 22). The large species produced fewer nests in 1988 (Table 3), possibly with smaller offspring (Table 23), but maintained the same number of cells per nest as in 1987, a non-drought year.

Hypothesis 2. Bees exposed to ELF EM fields, and bees not exposed, will make nest plugs of the same thickness and will devote the same proportion of nest space to reproduction.

No test of this hypothesis has been attempted as of this time. What follows is the text of the 1987 Annual Report, containing our thoughts on how the hypothesis will be tested:

We are currently trying to decide how best to analyze these data. The length of nest plugs will be tested first with M. inermis, since complete nests for this species usually have a solid, uninterrupted nest plug between the last reproductive cell and the nest opening. In contrast, M. relativa nests usually

have empty vestibular spaces between two or more nest plugs (Fig. 1), and are thus more complex to analyze. Nest plug lengths for $\underline{M}$. inermis are skewed in distribution (eg., Fig. 10). We hope to find a transformation that will normalize the distribution so that we can use a GLM analysis. If not, we will consider categorical modeling of different length classes (eg., 5-20mm, 20-35mm, 35-50mm, >50mm).

In analyzing the proportion of space devoted to reproduction, we wish to compare the sum of reproductive cell lengths with space in the nest used by the bee. Used space may not necessarily include the full nest depth. Basal spaces and indentations (Fig. 1) are first subtracted from total nest depth. The ratio of reproductive space to used space approaches 1.0 as the length of nest plugs and vestibular spaces decreases. We can test whether the distributions of this ratio for experimental and control areas are the same, using a Goodness of fit test.

Hypothesis 3. The number of leaves used to line a cell is unchanged when bees are exposed to ELF EM fields.

Although the number of leaves lining a cell is discrete data, treating it as if it were continuous, and using the GLM procedure on the log of leaves per cell, yielded normally distributed residuals for $\underline{M}$. inermis. Using a GLM, instead of a CATMOD analysis as originally planned, should reduce the minimum detectible difference between control and experimental areas.

GLM analyses were applied to four subsets of the $\underline{M}$. inermis data. First, all cells from nests with diameters greater than 9.5mm (bore size 7) were analyzed (Table 28). Second, cells from all cells for which offspring's sex was known were analyzed (Table 29). Third, only cells with male $\underline{M}$. inermis offspring (all diameters) were analyzed (Table 30). Finally, only cells with female $\underline{M}$. inermis offspring (all diameters) were analyzed (Table 31). The geometric mean (bore size 7 nests) was 11.8 leaves per cell. Male cells were constructed with more leaves (11.7) than female cells (10.6) on average (Tables 30-31). Coefficients of variation in the tests ranged from 7.4-8.5%, and 27 - 40% of the variability was explained by the models.

There was no significant effect of Exp, or Exp*Year in any of the models. This suggests that testing of the ELF antenna at half power during the summer of 1988 did not have any effect on the number of leaves per $\underline{M}$. inermis cells. Furthermore, there are no intrinsic differences between experimental and control areas contributing to variability in leaves per cell.

As expected, offspring's sex contributed significantly to variability in leaves per nest (Table 29). Date of nest initiation contributed significantly to number of leaves per cell in all four analyses. Cell order contributed

significantly to variability in leaves per nest when all cells, cells with offspring's sex, or just male cells were analyzed.

Assuming the same minimum sample sizes that were used to calculate minimum detectible differences between cell lengths in control and experimental areas for M. inermis (see above, p. 31), and $s^2 = 0.84$ (Table 28) we estimate that we can detect about a 15% difference in the log of number of leaves per cell with a power of 0.9 and $\alpha = 0.05$. This turns out to be either an increase of 5.5 leaves or a decrease of 3.7 leaves as compared with the current mean of 11.9 leaves. 124 is an underestimate of the sample sizes that we actually have. Sums over the four years for which we have data are 447 control cells and 2149 experimental cells. Using n=447, we can detect an increase of 2.2 leaves or a decrease of 2.1 leaves with the same power. Thus, in order to detect an effect of ELF EM fields on leaves per cell, experimental and control areas will have to differ by about twice the difference caused by offspring sex. It will take more than a change in sex ratio in order to detect differences between experimental and control sites in leaves per cell.

Hypothesis 4. The relative acceptability of nests oriented in a NS direction vs. nests oriented in an EW direction does not change when bees are exposed to ELF EM fields.

As explained in the methods section, at each site there are three sets of hutches. Each hutch set consists of two hutches in close proximity, one oriented N-S, and one oriented E-W. Nests on the N-S hutch have openings facing E or W, while nests on the E-W hutch have openings facing N or S. The directions used in this analysis refer to the direction of nest openings.

Each set of hutches is situated in a different location and has a different pattern of sun and shade during the day, and a different compliment of nearby flowering plants. These factors may be important in acceptance of nest opening direction by bees. Thus, we have analyzed nest orientation by hutch set at each site. Furthermore, since sample sizes are low at some hutches in some years, we have not tried to discriminate between nests oriented in four directions; rather we compare acceptance of nests oriented N or S vs. nests oriented E or W. Only data for M. relativa are analyzed, since sample size was very low most years for M. inermis at the control sites.

First we test whether both directions are equally acceptable using a Heterogeneity Chi-Square Goodness of Fit test (Table 32), with expected proportions of 50% N or S and 50% E or W. This test could not be used for years where fewer than 10 nests were constructed for a hutch set, because the expected frequencies would be less than 5. In cases where the test could be applied, the data were heterogeneous (except for the C5 W hutch). This means that in some years acceptance of nest directions was not different from

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a 50-50 distribution, while in other years the nests were different from 50-50 at a given hutch set.

As an alternative approach, we analyzed the same data with a Log-likelihood Ratio (G-test) Heterogeneity Contingency test (Table 33). This tests whether the pattern of nest preference (whatever the pattern) is the same for all years at a given hutch set. When the null hypothesis was accepted for all hutch sets at a site (everywhere except F1), the data was pooled over years at the hutch sets, and each hutch set was tested against the other hutch sets at that site, to test whether the pattern was consistent for the entire site.

The results indicate that there is often a consistent preference over the years at a given hutch set, but that in most cases the preference is different between hutch sets. If ELF EM fields are beginning to affect nest orientation preference, one might expect changes in nest orientation within a hutch set over the years at experimental but not control areas. Only two hutch sets at the F1 site have shown changes within a hutch set over the years. Overall, the heterogeneous results make it difficult, if not impossible, to attribute future changes in preference of nest orientation to effects of ELF EM fields, unless bees in the experimental area stop nesting in some directions altogether. The data to test Hypothesis 4 will continue to be available in case such a drastic change occurs, but we do not expect that nest orientation will be useful for detecting effects of ELF EM fields.

V. NEST ACTIVITY RESULTS

Sample sizes

Seven notebooks of nest activity data taken by five different observers from 1983 - 1986 have been transcribed to the computer. From these notebooks we have created a data set consisting of LO timings involved in cell cap construction. Those LO trips that were involved in nest plug construction, or in cell lining (see 1987 Annual Report) were not included in this data set. Number of bees for which we have LO trip durations at each of the four sites each year are presented in Table 34. Few bees were timed at the control sites during these early years.

During the 1987 - 1989 summer seasons, LO trips for at least 24 M. inermis individuals were timed at each of three of the sites (Tables 35, 36, 39). At the CL site in 1987, very few bees nested after July 10 (Fig. 6), so only 10 individual M. inermis were timed (Table 35). We were prepared to spend more time in 1988 timing bees early at the CL site to compensate for an expected late season decline in populations. However, a spring drought in 1988 prevented bloom of Hieracium aurantiacum at this site, so there was no early season activity of M. inermis at all. Very few individuals nested at the CL site after rain ended the drought in July. Thus, only 6 cells from 4 M. inermis individuals were timed at this site in 1988 (Table 36). In 1989, with the help of an additional assistant, and cooperation from the weather, we were able to time 20 bees at the CL site (Table 39).

This year, only the first eight trips involved in capping a cell were included in the analysis, in an effort to normalize the residuals of the GLM. In 1987, we rarely recorded the trip number of the first capping trip that was timed, so many of the 1987 timings had to be eliminated from the analysis. Tables 37, 38, and 39 give the actual numbers of cell caps and LO timings used in the present GLM analysis.

Averages from 3.7 to 5.6 LO trips per cell cap were used in the analyses (Tables 37, 38, 39). Usually all of these cell caps were constructed by different bee individuals. Our analysis assumes that the timings for a given cell cap are independent of the timings for other cell caps. The assumption may not be strictly true for a small number of cases (about 10%) in 1987 and 1988 in which the same bee was timed capping more than one cell. However, since only a small proportion of the timings fall in this category, we believe that the assumption of independence is not seriously violated.

Hypothesis 5. The duration of round leaf (LO) foraging trips remains the same when bees are exposed to ELF EM fields.

During the 1987 field season we noticed that LO trip durations increased with each successive trip after the bee lays her egg. In 1987, however, we did not keep track of which LO trips in the capping sequence were being timed. Occasionally, we could deduce which trip had started the timings. Just before collecting the first LO in the cap, after laying her egg, the female makes a series of very rapid flights in and out of the nest. Undergraduate observers refer to this behavior as "spazzing". Where rapid flights in and out of the nest, without a cargo, appear at the beginning of a series of 1987 LO timings, we have assumed that the first LO trip for the cell has been timed.

In 1988 we recorded the actual trip number for 73% of the capping sequences that were timed. In 1989, we were even more diligent, recording actual trip numbers for every cell cap timed. In our analyses, we discovered that residuals were normal only when we used a Log(Log) transformation of LO trip durations, and only when we restricted the analysis to trips 1-8.

The results of a GLM analysis of 1989 Log(Log) LO durations are presented in Table 40. The mean duration was 21 seconds, less than the 36 seconds reported in 1987 and 24 seconds reported in 1988. (The earlier means were geometric means, while this year's mean is derived from the Log of the geometric mean, which for skewed data is expected to be less than the geometric mean.) The coefficient of variation was not much changed from last year (17.8 in 1989, 17.6 in 1988; 18.6 in 1987). The proportion of the variability explained by the model is low: $r^2 = 0.14$ in 1989, $r^2 = 0.28$ in 1988, $r^2 = 0.15$ in 1987. As in previous years, experimental vs. control areas did not contribute significantly to the variability. Since this is the first year that the antenna was on full power most of the summer, these negative results suggest that the antenna is not affecting LO trip durations.

As expected, trip rank contributes significantly to variability in LO trip durations. Time of day did not contribute to variability in 1987 but did in 1988 and 1989 (see 1987 and 1988 Annual Reports) suggesting that temperature or other weather parameters contribute to variability in LO duration. Date of the timing was not significant in 1987 or 1989, but was in 1988.

When only 1989 timings are analyzed, differences between observers are not a significant contributor to variability. This result can be attributed to the training video created last summer, combined with greater consistency between observers in starting timings with the first or second LO trip in a sequence, and greater consistency between observers in the proportion of timings made at each site.

Combining data from 1987-1989 in one analysis proved more difficult, because of the more reliable trip ranks for 1988 and 1989. Also, very few trips after the 8th LO were timed in 1989; whereas, many trip ranks in 1987 and 1988 were greater than the 8th LO, or were unknown. Only 1987 and 1988 trips for which trip rank was known, and was less than 8 were included in this analysis. The results (very similar to 1989 results) are presented in Table 41. Neither Exp, Year, or Exp*Year contributed significantly to the variance, though trip rank and observer did. When 1987 and 1988 data were included in the analysis, there was no significant contribution of date or time of day to variability in LO duration. The overall mean LO duration was 22 seconds. 19% of the variance was explained by the model; more than for 1989 data alone (Table 40). Lack of significance for Exp*Year confirms the result from 1989 data alone that the ELF EM fields are not affecting LO trip durations.

In 1987, bees averaged 11 sec. faster at the control sites (31 sec.) than at the experimental sites (42 sec.). There was a greater difference between the two control sites than between the two experimental sites. (C5=34, CL=23, difference=11 sec.; F1=39, F2=46, difference=7 sec.) The same pattern was seen in 1988, when bees averaged 7 sec. faster at the control (20 sec.) than the experimental (27 sec.) sites (C5=21, CL=17, difference=4; F1=28, F2=26, difference=2 sec.) Differences were similar in 1989. Control sites averaged 19.5 sec., experimental sites 21.7 sec.; 2.2 sec. difference (C5=19, CL=21, difference=2; F1=23, F2=20, difference=3). These differences are not significant, however, and have been decreasing over the years.

The data prior to 1987 were analyzed (in previous reports, repeated here) using the same GLM procedure. Because so few individuals were timed in any given year, we combined all LO trip durations over years, and did not test for differences between years. Although control sites were under-represented, we present these data for comparison with 1987 because of the trends suggested. As in 1987, LO trip durations at control sites averaged 12 seconds faster than at experimental sites. Unlike 1987 and 1988, the difference between sites was greater for experimental than control areas, although average LO trip durations had the same rank order of sites in all years (C5=29, CL=26, difference=3; F1=37, F2=48, difference=11 sec.). In the GLM procedure (Table 42), neither experimental vs. control areas, sites nested in areas, nor time of day contributed significantly to the variance in LO duration. Trip order, observer, and date were significant. These results correspond well with the 1987 - 1989 data.

Weather data, and presence or absence of ELF EM fields during the LO trip are variables being added to our nest activity SAS data set, so we can incorporate them into our model of nest activity in the future. This process was slowed when our data manager left in April for a new job. A graduate student has been trained to handle data management, but she has not had

time to work on weather and EM fields this year, because her commitment to the ELF project is less than that of the former data manager.

Based on our performance during the 1987-1989 seasons (Table 41), with a combined sample size of at least 500 trips for the two control sites, and an error mean square for Exp of 0.765, we should be able to detect an increase from 22 sec. to 41 sec. or a decrease to 13 sec. at the experimental area with α = 0.05 and a power of 0.9. This is comparable to last year's results, and is well within the observed range of LO durations.

VI. EMERGENCE RESULTS

For the most part, both species of <u>Megachile</u> in our study are univoltine, having only one generation per year. There have been a few exceptions: In <u>M</u>. <u>relativa</u> nests, 2-10% of all <u>M</u>. <u>relativa</u> and <u>Coelioxys</u> sp. emergences occur in August and September (Table 43). Far fewer instances of bivoltinism occur in <u>M</u>. <u>inermis</u> nests (Table 44). Early emergences do not overwinter, and are not included in the analysis described below.

<u>Hypothesis 6</u>. **Overwintering mortality of megachilid bees is unchanged by exposure to ELF EM fields.**

Prior to emergence as an adult in the Spring, <u>Megachile</u> are subject to a variety of sources of mortality. The egg may fail to hatch, or the larva may die of unknown causes during the summer. The prepupa may die during the winter. The pupa may fail to eclose in the spring. A number of parasites may attack the <u>Megachile</u> egg, larva, or pupa at various times in its development. Parasites include the cuckoo bees, <u>Coelioxys moestus</u> Cresson on <u>M</u>. <u>relativa</u> and <u>C</u>. <u>funeraria</u> Smith on both <u>Megachile</u> spp.; the fly <u>Anthrax irroratus irroratus</u> Say; chalcid and leucopsid wasps.

The percent mortality due to various causes is presented by site and year for <u>M</u>. <u>relativa</u> and for <u>M</u>. <u>inermis</u> in Tables 45 and 46 and Figs. 11 and 12. These tables show that pre-overwintering mortality (mortality of eggs and larvae) was greater in 1987 than in previous years (for <u>M</u>. <u>relativa</u>), and even greater in 1988 (for both species). This is probably due in part to the change in protocol in 1987, leaving nests to overwinter in the field rather than bringing them to Channing. Weather patterns are undoubtedly also involved. High mortality in 1988 nests was probably due to dry, hot summer weather (pre-overwintering mortality), and to unusually cold spring weather (overwintering mortality). Numerous summer rainfalls may have caused higher pre-overwintering mortality in 1987 as compared to earlier years. Similarly, proportion of adults emerging was particularly low for 1988 nests. The proportion of cells with prepupal mortality (i.e., the overwintering mortality) was low at all sites and in all years, varying between 0.014 and 0.134 (<u>M</u>. <u>relativa</u>) and from 0 to 0.164 (<u>M</u>. <u>inermis</u>) and showing no obvious patterns between sites.

There are several ways that one can measure overwintering mortality, and several problems that must be dealt with in analyzing it. First, we equate overwintering mortality with the prepupal stage, but actually the prepupa lasts for a longer time than just the winter. The prepupal stage begins several weeks after the egg is laid, when the larva has finished eating its provisions. The prepupa defecates shortly after molting, and then spins a silken cocoon for overwintering that is surrounded by fecal pellets. Thus the prepupal stage may begin as early as mid-summer. It lasts until pupation in the spring. This

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probably occurs typically in mid to late May, although we have not opened cells to find out, because this is likely to increase mortality. In 1989, the prepupal stage probably lasted into June, due to cool weather, and a change in protocol to a shady outdoor emergence site. Emergence usually begins in June, but in 1989 emergence of nests constructed in 1988 was delayed until July. Figs. 13 and 14 compare emergence of 1987 and 1988 nests in 1988 and 1989 respectively.

There is no way to separate prepupal mortality that occurs during the winter from prepupal mortality that occurs in summer, fall or spring. 1987 and 1988 nests were left at the sites where they were constructed during the entire prepupal stage except for the last few weeks, when nests were returned to the lab for nest architecture measurements. Thus, the effects of ELF EM fields on prepupal mortality any time before May are tested by our protocol.

Prior to 1989, pupation and emergence took place in the lab where handling, indoor microclimate, and 60 hz EM fields could affect pupal and adult mortality. Starting in 1989, the effects of 60 hz EM fields were minimized by moving emergence of all cells to an outdoor holding site. We have no way of knowing how many adult bees would have successfully emerged at the study sites, but the number of cells that survive past the prepupal stage provides an upper limit. Therefore, we combine pupae, adults that die in the cocoon, and adults that successfully emerge, into one "post-overwintering" category.

The prepupal stage has the longest duration of all the developmental stages of these univoltine species. However, mortality is greater in the pre-overwintering egg and larval stages. Mortality of these early stages seem to show differences between years and sites (Tables 45, 46, Figure 11, 12) that could make it difficult to detect differences due to ELF EM fields. Therefore, we propose restating our hypothesis as: **Given that a bee survives to the prepupal stage, the probability that it will not survive past the prepupal stage does not change in the presence of ELF EM fields.** Thus, we analyze proportion of mortality in the prepupal stage, calculated as the number of cells with a dead prepupa divided by the sum of cells with prepupae or post-overwintering bees. Cells containing egg and larval mortality are not included.

Parasites present another problem. It is easy to distinguish adult and pupal Megachile from adult and pupal parasites. However, we are unable to distinguish prepupae of Megachile from prepupae of the cuckoo bee, Coelioxys (also in the Megachilidae). The Coelioxys larva kills its host larva or egg, and feeds on the provisions in the cell. Like the host bee, Coelioxys overwinters in the prepupal stage. When testing the hypothesis above, the number of cells with dead prepupae should be reduced by the percentage of cells that are parasitized by Coelioxys. We can estimate percent parasitism of

prepupae from the proportion of adults that are parasites. This assumes that there is no differential mortality of parasites in the prepupal stage as compared with the adult stage.

In our first attempts to analyze prepupal mortality, however, we have not tried to separate Megachile and Coelioxys data. Rather, we assume that both genera are affected in the same ways, if at all, by ELF EM fields. This assumption is more likely to be true for two bee species in the Megachilid family, than for a bee and a fly or wasp parasite. We calculated proportion of prepupal mortality for each site and year by dividing the number of cells containing dead prepupae (X) by all cells with Megachile or Coelioxys prepupae or post-overwintering stages (n).

Neither Exp nor Exp*Year contributed significantly to variance in proportion of prepupal mortality for either M. relativa (Table 47) or M. inermis (Table 49). This suggests that testing of the ELF antenna at half power during the winter of 1988-89 did not affect overwintering mortality. Site[exp] was significant for M. inermis. Year was significant for both species, with 1988 differing from previous years for M. relativa (Table 48), and all years differing from each other for M. inermis (Table 50). Coefficients of Variation were 26.2 % for M. relativa and 8.3 % for M. inermis. The r^2 were 0.78. and 0.98 respectively. The original proportions varied from 0.015 (F1, 1988) to 0.217 (CL, 1988) for M. relativa, and from 0.0 (CL, 1985, 1986) to 0.204 (C5, 1988) for M. inermis.

In the 1987 annual report, a jackknifing technique was used to calculate the minimum detectible difference in prepupal mortality. In the simulation, the 1985 - 1987 data were used to create a fourth year of simulated data for each site, based on the average of the three years for which data were available. The new proportion of prepupal mortality was randomly generated from a Binomial distribution with n=the average number of prepupal, pupal, and adult cells over the three years, and p=the mean proportion of prepupal mortality over the three years. At the experimental sites, we multiplied p by a factor to simulate the possibility that the ELF EM fields increased prepupal mortality. 100 realizations of this simulation were made for each of three multiplication factors. The ANOVAs tested for the effects of Year, Site, and a new variable called ELF, which was set equal to 0 for all sites and years except the Experimental sites in the simulated year. The Freeman and Tukey arcsine transformation was used in these simulations.

When the multiplication factor was 2, the ELF variable was never significant, indicating that we would not be able to detect a doubling of percent prepupal mortality due to the ELF fields. When percent prepupal mortality was increased 2.5 fold, the ELF variable was significant at the 0.05 level in 64 out of 100 realizations. However, when percent prepupal mortality was tripled, the ELF variable was significant at the 0.05 level in all

100 realizations of the ANOVA, and was significant at 0.01 in 78 of the 100 realizations, indicating that we should be able to detect a tripling in prepupal mortality. A three fold increase in percent prepupal mortality at the experimental sites is a change from approximately 5 or 6% to 15 or 18%. It seems reasonable to be able to detect a change of this magnitude, since the original prepupal mortality was very low.

We have not repeated the jackknifing technique with 1985-1988 data, but have done a few preliminary realizations with the following modifications from last year's analysis: First, we created a fifth year of simulated data for each site based on average proportion mortality for 1985-1987. This assumes that the high 1988 prepupal mortality will not be repeated in the future. Total number of nests for the hypothetical fifth year was based on the average of all four years, 1985-1988. Instead of using a new variable, ELF, we look for a significant Exp*Yr interaction, where the error term was the Site[exp] mean square.

We tried four realizations of this simulation using a multiplication factor of 3 for the experimental sites in the fifth year, and four realizations using a multiplication factor of 5. While differences between years were always significant in these analyses, the Exp*Yr interaction was never significant. This suggests that it will be much more difficult to detect a significant Exp*Yr interaction than it was to detect significance of an ELF variable in last year's analysis. The ELF term is tested with the overall model error term, and does not take into account differences between sites nested in experimental and control areas. This year's jackknifing procedure is more conservative. However, since it will probably not be possible to detect differences in overwintering mortality by testing for Exp*Yr significance, in next year's analysis we will use an ELF variable, as described above, to test for effects of ELF EM fields.

Nests constructed in 1988 were the first to overwinter in the same orientation as they were constructed. We analyzed 1988 data, adding the additional variable "direction", indicating whether the nest overwintered along a north-south axis, or along an east-west axis. (It is not necessary to separate nests by hutch set, as in our analysis of acceptability of nest orientations, because nests from all hutch sets at a site were overwintered in the same box.) We are curious to determine whether direction of the nest contributes significantly to prepupal mortality, particularly at the experimental sites where nests are exposed to ELF magnetic fields that might differentially affect prepupae oriented in particular directions (see introduction, p. 9). Thus, we are looking for a significant effect of Direction*Exp.

The interaction was not significant for M. relativa (Table 51, Fig. 15) but was for M. inermis (Table 52). Nests oriented EW had similar mortality at all

sites (Fig. 16). Nests oriented NS had higher mortality than nexts oriented EW at the control sites, but lower mortality than nests oriented EW at the experimental sites. This is our first indication that ELF EM fields may have an effect on leaf-cutter bees, but it is opposite from the increased mortality at the experimental sites that one might expect. If this result is related to the ELF antenna, it suggests that ELF EM fields ameliorate some natural increase in mortality when prepupae are oriented parallel to the earth's magnetic field. This seems far-fetched, but not impossible. The 1988 results for M. relativa, while not significant, are consistent with this hypothesis (Fig. 15). If the results are caused by ELF EM fields, we expect the 1988 pattern of prepupal mortality with direction and site to occur in future years.

In previous years, when nests overwintered in Channing (1985-1986), or did not overwinter in the same orientation as they were constructed, patterns of prepupal mortality were different with respect to direction than in 1988 (Figs. 15, 16), but are not strictly comparable with the 1988 data.

VII. SUMMARY

Studies of the effects of high voltage transmission lines and magnetic fields in honeybees suggest several ways that solitary megachilid bees might be affected by ELF electromagnetic fields. In particular, honeybees show greater levels of activity, reduced reproductive output, lower overwintering survival and modifications of nest structure in response to high voltage transmission lines. In addition, honeybees can detect magnetic fields and may use them in orientation. ELF EM fields may affect megachilid bees in similar ways.

Megachilid bees are particularly well suited for this study. Their investment per offspring and reproductive output per nest are easy to measure because they provide each offspring with a discrete cell, and because they readily nest in artificial nests. Three types of data have been gathered in past years: nest architecture, nest activity, and emergence/mortality.

Two abundant species at the experimental and control sites, both in the genus _Megachile_, are the focus of our analysis. These species differ in size and degree of sexual dimorphism. Thus, they may be impacted differently by ELF EM fields.

Four hypotheses regarding the impact of ELF EM fields on nest architecture are being tested:

Hypothesis 1. The average size (length and volume) of cells for each offspring, and/or the average number of cells produced per nest is unchanged by exposure to ELF electromagnetic fields.

Hypothesis 2. Bees exposed to ELF EM fields, and bees not exposed, will make nest plugs of the same thickness and will devote the same proportion of nest space to reproduction.

Hypothesis 3. The number of leaves used to line a cell is unchanged by exposure to ELF EM fields.

Hypothesis 4. The relative acceptability of nests oriented in a NS direction vs. nests oriented in an EW direction does not change when bees are exposed to ELF EM fields.

Nest architecture data for both _M_. _relativa_ and _M_. _inermis_ nests constructed in 1985-1988 have been analyzed. These data suggest that, prior to the ELF antenna becoming fully operational, there are no significant differences between experimental and control areas in cell length. Similarly, there is no significant interaction between experimental vs. control areas and

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year, in cell lengths. This means that prior to operation of the ELF antenna, cell lengths in both areas are affected equally by differences between years, or are not affected by years. Thus, we should be able to detect effects of ELF EM fields on cell lengths and volumes by a significant interaction term when the antenna is operational. The minimum number of nests that we have collected at the control sites is sufficient to detect a 9% change in mean cell length for M. relativa (mean = 11.1mm) and a 14% change in mean cell length for M. inermis (mean = 15.7mm) with a power of 0.9 and an α of 0.05.

Number of cells per M. relativa nest were significantly different between sites nested in experimental and control areas, and between years. Number of cells per M. inermis nest is significantly greater for experimental than control areas, but Year and Exp*Year are not significant. Thus there are intrinsic differences in the distribution of cells per nest between sites and areas for these species, but no effects of ELF EM fields at 50% power.

Differences between experimental and control areas, and the interaction between year and experimental vs. control areas did not contribute significantly to variability in number of leaves per M. inermis cell (Hypothesis 3). Thus half power ELF EM fields do not affect leaves per cell for this species. We should be able to detect a 2.2-5.5 leaf change in the current mean of 11.8 leaves per cell if full power ELF EM fields have an impact on this variable.

Nest entrance orientation (hypothesis 4) was highly variable for M. relativa between hutch sets at a given site, between sites, and between experimental and control areas. Because of this heterogeneity, the data are not likely to be useful for detecting effects of ELF EM fields.

We have not yet analyzed the data to test hypotheses 2.

One hypothesis regarding nest activity is being tested:

Hypothesis 5. The duration of round leaf (LO) foraging trips remains the same when bees are exposed to ELF EM fields.

Changes in protocol begun in 1987 have greatly increased the number of bees timed for the duration of round-leaf collecting trips. However, much of the data collected in 1987 was not included in our current analysis, because trip rank was not available. This year's analysis was restricted to the first 8 leaf collecting trips after the egg is laid. Experimental vs. Control areas did not contribute significantly to the variance in LO trip duration for M. inermis. Thus, ELF EM fields at full power do not affect LO trip durations. The trip rank number in a capping sequence is a significant covariate with LO trip duration. If sample sizes in the future are comparable with those from 1987-89, we should be able to detect a 1.9 fold increase in LO duration (from 22 to 41

seconds) with a power of 0.9 and $\alpha = 0.05$. This magnitude of change is possible if bees are disoriented by ELF EM fields.

One hypothesis concerning emergence and mortality data has been tested:

Hypothesis 6. Overwintering survival of megachilid bees is unchanged by exposure to ELF fields.

Overwintering mortality takes place when the bee is in the prepupal stage. Because of the effects of microhabitat and year on pre-overwintering (larval) mortality, it was decided to eliminate cells with this mortality from our analysis. Thus our hypothesis has been restated as: Given that a bee survives to the prepupal stage, the probability that it will not survive past the prepupal stage does not change in the presence of ELF EM fields. Thus, we calculate proportion of mortality in the prepupal stage as the number of cells with a dead prepupa divided by the sum of cells with prepupae, pupae, dead adult, or emerging adult bees. Mortality of the parasitic cuckoo bee, Coelioxys, is included in the analysis, since we cannot distinguish the two bee species until the pupal stage.

No significant differences between experimental and control areas were found in a preliminary analysis of 1985-1988 M. relativa and M. inermis prepupal mortality. Mortality was significantly higher for 1988 nests than previous years, probably due to either effects of drought or the cold spring in 1989. However, when 1988 nests were separated by orientation, NS oriented nests had significantly lower mortality at experimental sites than at control sites for M. inermis. This effect may be caused by ELF fields, because 1988 was the first year that the antenna was tested during the winter. 1988 was also the first year that the nests were overwintered in the direction that they were constructed. Only if this pattern is repeated in the next few years will ELF EM fields be implicated as the cause of reduced mortality in NS oriented bees.

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TABLE 1. Bore size categories and diameter of drill bits associated with each category. Number of x's indicate relative use by the two <u>Megachile</u> species.

Size	Diameter, mm	Used by M. relativa	Used by M. inermis
6*	4.4		
2*	5.2	xx	
4	5.5,6.0	xxx	
5*	7.2	xx	x
3*	9.4		xx
7	11.0		xxx

* Bore sizes used before 1987 only.

TABLE 2

ELF antenna operations, and season when data was (will be) collected.

Year of Nest Contruction or Overwintering	1985 Su	1985 W	1986 Su	1986 W	1987 Su	1987 W	1988 Su	1988 W	1989 Su	1989 W	1990 Su	1990 W	1991 Su	1991 W	1992 Su	1992 W	End
ELF EM Field	0	0	10%	0	10%	10%	50%	50%	100%	100%	100%	100%	100%	100%	100%	100%	
	"NOT ON"										"ON"						
Activity Data	—		—		1987		1988		1989		1990		1991		—		
Architecture Data	1987		1987		1988		1989		1990		1991		1992 →		1992 →		
Overwintering (Emergence Data)		1987		1987		1988		1989		1990		1991		1992 →			

TABLE 3: Number of nests for which we have data on complete cell lengths by site for M. relativa and M. inermis at each site . Numbers in parenthesis indicate number of hutches out of six total with five or more nests of a given species.

Site Year	Control Sites		Test Sites	
	Camp 5	County Line	Ford 1 (North)	Ford 2 (South)
	M. relativa			
1985	48 (5)	67 (6)	81 (5)	85 (6)
1986	41 (6)	49 (5)	39 (4)	81 (5)
1987	77 (5)	49 (5)	76 (4)	47 (5)
1988	104 (6)	82 (6)	94 (6)	74 (6)
1989*	62 (6)	40 (5)	33 (2)	63 (6)
	M. inermis			
1985 nests measured	23 (3)	17 (2)	159 (6)	88 (6)
nests constructed**	26	18	212	121
1986	15 (1)	2 (0)	40 (3)	65 (4)
1987	56 (3)	25 (3)	122 (5)	108 (6)
1988	38 (4)	8 (0)	59 (2)	140 (5)
1989*	101 (6)	19 (2)	148 (6)	236 (6)

* Approximate numbers; Complete nests only; not yet measured

** Some 1985 nests were not measured because they were used in a study of diapause. I do not have these nests, nor do I have the data from the diapause study.

TABLE 4: GLM of cell lengths for all cells from 1985 - 1988 M. relativa nests.

CELL LENGTHS

Source of variation	df	SS	F	P>F
Year	3	73.85	2.14	0.1653
Diameter	1	84.04	90.36	0.0001***
Exp	1	21.40	1.29	0.3741
Site[Exp]	2	33.23	17.86	0.0001***
Exp*Year	3	15.96	0.32	0.8152
Complete vs. incomplete	1	8.86	9.52	0.0020*
Measurer[Yr]	9	103.56	12.37	0.0001***
Cell order	1	63.81	68.61	0.0001***
Cells per nest	1	9.89	10.64	0.0011*
Date	1	116.51	125.27	0.0001***
Model	23	712.45	30.98	0.0***
Error	3437	3197.98		

$\bar{X}$ = 11.0mm   CV = 8.8 r^2 = 0.18

TABLE 5: GLM of cell length for cells from 1985 - 1988 M. relativa nests with bore diameters between 5.0 and 7.0mm.

CELL LENGTHS

Source of variation	df	SS	F	P>F
Year	3	46.44	2.09	0.1719
Diameter	1	21.34	22.97	0.0001***
Exp	1	23.05	1.52	0.3430
Site[Exp]	2	30.35	16.33	0.0001***
Exp*Year	3	13.57	0.30	0.8283
Completevs. incomplete	1	7.55	8.13	0.0044*
Measurer[Yr]	9	66.67	7.97	0.0001***
Cell order	1	54.26	58.39	0.0001***
Cells per nest	1	12.07	12.99	0.0003***
Date	1	115.27	124.06	0.0001***
Model	23	516.43	24.17	0.0***
Error	3051	2834.89		
$\bar{X}$ = 11.1mm	CV = 8.7	r^2 = 0.15		

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TABLE 6: GLM of cell lengths for 1986 - 1988 M. relativa nests; leaves per cell and all diameters included.

CELL LENGTHS

Source of variation	df	SS	F	P>F
Year	3	15.51	0.82	0.5218
Diameter	1	12.53	14.51	0.0001***
Exp	1	13.93	2.29	0.2692
Site[Exp]	2	12.15	7.03	0.0009**
Exp*Year	2	0.08	0.01	0.9931
Complete vs. incomplete	1	0.42	0.49	0.4863
Measurer[Yr]	7	44.02	7.28	0.0001***
Cell order	1	9.68	11.21	0.0008**
Cells per nest	1	11.09	12.84	0.0004***
Date	1	51.79	59.97	0.0001***
Leaves per cell	1	4.63	5.36	0.0207*
Model	22	193.33	10.18	0.0001***
Error	1465	1265.18		

$\bar{X}$ = 11.2mm   CV = 8.3 r^2 = 0.13

TABLE 7: GLM of cell lengths for 1985 - 1988 M. relativa nests; offspring's sex known and all diameters included.

CELL LENGTHS

Source of variation	df	SS	F	P>F
Year	3	9.21	0.67	0.5897
Diameter	1	41.76	53.66	0.0001***
Exp	1	16.03	5.72	0.1392
Site[Exp]	2	5.60	3.60	0.0276
Exp*Year	3	3.39	0.40	0.7684
Complete vs. incomplete	1	10.93	14.04	0.0002***
Measurer[Yr]	9	41.04	5.86	0.0001***
Cell order	1	5.70	7.32	0.0069**
Cells per nest	1	14.40	18.51	0.0001***
Date	1	33.94	43.60	0.0001***
Sex	1	67.09	86.21	0.0001***
Sex*year	3	5.08	0.37	0.7757
Sex*exp	1	1.77	0.63	0.5095
Exp*sex*year	3	0.75	0.09	0.9591
Model	31	438.63	18.18	0.0***
Error	1475	1147.93		

$\bar{X} = 11.1mm$ $CV = 7.9$ $r^2 = 0.28$

TABLE 8: GLM of cell lengths for 1985 - 1988 <u>M</u>. <u>relativa</u> nests; male offspring and all diameters included.

CELL LENGTHS

Source of variation	df	SS	F	P>F
Year	3	14.53	1.15	0.3794
Diameter	1	40.70	52.16	0.0001***
Exp	1	10.45	4.23	0.1761
Site[Exp]	2	4.95	3.17	0.0424*
Exp*Year	3	6.87	0.93	0.5567
Complete vs. incomplete	1	5.70	7.30	0.0070*
Measurer[Yr]	9	37.77	5.38	0.0001***
Cell order	1	3.12	4.00	0.0456*
Cells per nest	1	12.46	15.97	0.0001***
Date	1	27.55	35.30	0.0001***
Model	23	211.78	11.80	0.0***
Error	1213	946.58		

$\bar{X}$ = 10.9mm   CV = 8.1 r^2 = 0.18

TABLE 9: GLM of cell lengths for 1985 - 1988 M. relativa nests; female offspring and all diameters included.

CELL LENGTHS

Source of variation	df	SS	F	P>F
Year	3	2.95	0.46	0.7151
Diameter	1	0.09	0.13	0.7235
Exp	1	5.81	7.38	0.1131
Site[Exp]	2	1.58	1.09	0.3376
Exp*Year	3	0.84	0.36	0.7940
Complete vs. incomplete	1	2.98	4.12	0.0435*
Measurer[Yr]	9	19.13	2.94	0.0025*
Cell order	1	3.48	4.81	0.0292*
Cells per nest	1	4.04	5.59	0.0188*
Date	1	9.45	13.07	0.0004**
Model	23	78.37	4.72	0.0001***
Error	246	177.77		

$\bar{X}$ = 11.8mm   CV = 7.2 r^2 = 0.31

TABLE 10: Summary of models of <u>M</u>. <u>relativa</u> cell length, comparing the 1988 Annual Report (85-87 nests) with the current report (85-88 nests).

| | # Cases with Variable Significant/ Total # Cases Tested | | | |
| | All Cells | | Bore Diameters 5.0 to 7.0mm | |
	85-87	85-88	85-87	85-88
Year	1/4	0/5	2/4*	0/3
Diameter	4/4*	4/5*	4/4*	3/3*
Exp	0/4	0/5	0/4	0/3
Site[Exp]	2/4*	4/5*	2/4*	3/3*
Exp*Year	0/4	0/5	0/4	0/3
Complete vs. incomplete	3/4*	4/5*	2/4*	2/3*
Measurer[Yr]	4/4*	5/5*	4/4*	3/3*
Cell order	4/4*	5/5*	4/4*	3/3*
Cells per nest	0/4	5/5*	1/4	3/3*
Date	1/1*	5/5*	1/1*	3/3*
Leaves per cell	0/1	1/1*	0/1	1/1*
Sex	1/1*	1/1*	1/1*	1/1*
Sex*year	0/1	0/1	0/1	0/1
Sex*exp	na	0/1	na	0/1
Exp*sex*year	0/1	0/1	0/1	0/1
Range of CVs	8.1-8.9	7.2-8.8	7.8-8.7	7.9-8.8
Range of r^2	.11-.28	.13-.31	.11-.26	.14-.27

* Contributed significantly to variability in cell length in most analyses.

TABLE 11: Differences between observers in mean cell lengths (M. relativa, bore diameters between 5.0 and 7.0mm only).

Measurer	Mean Cell Lengths m m	No. Cells Measured
ER (1985)	10.90	234
ND (1985)	10.59	267
KS (1985)	10.98	208
JZ (1986)	11.11	190
KS (1986)	11.08	201
LS (1986)	10.65	160
MS (1986)	10.92	84
KS (1987)	11.33	390
LS (1987)	11.05	129
VS (1987)	11.31	464
BZ (1988)	10.86	186
KS (1988)	11.29	220
VS (1988)	11.03	342

TABLE 12: Mean cell length by cell order in the nest, 1985 -1988, all years and sites combined.
Basal cell = C1.

Cell Order	M. relativa			M. inermis		
	N	$\overline{X}$	SD	N	$\overline{X}$	SD
C1	925	11.3	1.1	896	16.0	1.7
C2	752	11.0	1.1	811	15.7	1.7
C3	569	11.0	1.0	732	15.5	1.7
C4	403	10.9	1.0	611	15.5	1.4
C5	288	10.8	1.0	452	15.6	1.4
C6	211	10.9	0.9	208	15.4	1.3
C7	158	10.7	1.0	52	15.1	1.4
C8	90	10.7	1.1	5	14.7	0.9
C9	55	10.6	0.8			
C10	15	10.3	0.8			
C11	4	10.5	0.9			
C12	1	10.2				

TABLE 13: Summary of models of M. relativa cell volumes from the 1987 Annual Report (85-86 nests). 1987-88 nests have not yet been included in the analysis.

| | # Cases with Variable Significant/ Total # Cases Tested | | | |
| | All Cells | | Bore Size 4 | |
	85-87	85-88	85-87	85-88
Year	1/3	na	1/1*	na
Diameter	4/4*	na	1/1*	na
Exp	0/4	na	0/1	na
Site[Exp]	2/4*	na	1/1*	na
Exp*Year	0/3	na	0/1	na
Complete vs. incomplete	1/4	na	1/1*	na
Measurer	4/4*	na	1/1*	na
Cell order	4/4*	na	1/1*	na
Cells per nest	1/4	na	0/1	na
Date	1/1*	na	na	na
Leaves per cell	1/1*	na	na	na
Sex	1/1*	na	na	na
Range of CVs	9.0-9.9	9.6		
Range of r^2	.87-.89	.73		

* Contributed significantly to variability in cell length in most analyses.

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Table 14: Sex Ratio by Site and Year for M. relativa.

	1985			1986		
Site	Males	Females	Ratio	Males	Females	Ratio
C5	96	9	10.7	69	23	3.0
CL	130	48	2.7	76	9	8.4
F1	262	41	6.4	94	18	5.2
F2	128	31	4.1	205	32	6.4
Total	616	129	4.8	444	82	5.4

	1987			1988		
Site	Males	Females	Ratio	Males	Females	Ratio
C5	186	59	3.2	70	25	2.8
CL	56	24	2.3	23	7	3.3
F1	186	59	3.2	111	12	9.3
F2	38	7	5.4	32	9	3.6
Total	487	157	3.1	236	53	4.5

TABLE 15: Two-Way, Mixed Model ANOVA partitioning the variance in cell length within- and between- measurer.

CELL LENGTHS

Source of Variance	DF	MS	F	P>F
Between Measurers	3	9.587	65.39	0.0001***
Between Cells	38	7.540	51.42	0.0000***
Within Measurer (Error)	355	0.147		

$\bar{X} = 10.5$mm   CV = 3.6   r2 = 0.86

Between Measurers	$s^2+2.55s^2_{mc}+39(2.55)s^2_m$	0.095	9.8%
Between Cells	$s^2+2.55s^2_{mc}+4(2.55)s^2_c$	0.725	75.0%
Within Measurer Error)	$s^2+2.55s^2_{mc}$	0.147	15.2%

TABLE 16: GLM of cell length for cells from 1985 - 1988 M. inermis nests with bore diameters greater than 9.5mm.

CELL LENGTHS

Source of variation	df	SS	F	P>F
Year	3	17.85	0.05	0.9855
Diameter	1	8.34	4.96	0.0260*
Exp	1	27.35	1.32	0.3691
Site[Exp]	2	41.37	12.30	0.0001***
Exp*Year	3	3.06	0.05	0.9820
Complete vs. incomplete	1	43.13	25.65	0.0001***
Measurer[Yr]	8	1013.83	75.38	0.0***
Cell order	1	80.38	47.81	0.0001***
Cells per nest	1	175.67	104.49	0.0001***
Date	1	19.20	11.42	0.0007**
Leaves per cell	1	52.50	31.23	0.0001***
Bore Depth	1	10.12	6.02	0.0142*
Bore Depth*Year	3	20.31	0.05	0.9826
Diameter*Bore depth	1	11.09	6.60	0.0103*
Diameter*Year* Bore Depth	3	22.10	0.06	0.9803
Diameter*Year	3	19.81	0.05	0.9832
Model	34	2089.41	36.55	0.0***
Error	2553	4292.18		

$\overline{X}$ = 15.7mm   CV = 8.2 r^2 = 0.33

TABLE 17: GLM of cell lengths for 1985 - 1988 <u>M</u>. <u>inermis</u> nests; offspring's sex known and all diameters included.

CELL LENGTHS

Source of variation	df	SS	F	P>F
Year	3	3.69	0.02	0.9967
Diameter	1	6.46	5.41	0.0202*
Exp	1	3.43	0.26	0.6616
Site[Exp]	2	26.5	111.10	0.0001***
Exp*Year	3	5.25	0.13	0.9327
Complete vs. incomplete	1	4.95	4.14	0.0420*
Measurer[Yr]	8	572.68	59.92	0.0***
Cell order	1	0.21	0.18	0.6748
Cells per nest	1	134.61	112.68	0.0001***
Date	1	1.33	1.12	0.2909
Leaves per cell	1	29.10	24.36	0.0001***
Sex	1	89.93	75.28	0.0001***
Year*Sex	3	3.70	0.02	0.9967
Exp*Sex	1	0.03	0.00	0.9650
Year*Exp*Sex	3	14.27	0.36	0.7931
Bore Depth	1	6.68	5.59	0.0182*
Bore Depth*Year	3	4.21	0.02	0.9960
Diameter*Bore Depth	1	7.63	6.39	0.0116*
Diameter*Year* Bore Depth	3	5.04	0.02	0.9947
Diameter*Year	3	4.14	0.02	0.9961
Model	42	1430.19	28.50	0.0***
Error	1302	1555.44		

$\bar{X}$ = 15.8mm   CV = 6.9 r^2 = 0.48

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TABLE 18: GLM of cell lengths for 1985 - 1988 M. inermis nests; male offspring and all diameters included.

CELL LENGTHS

Source of variation	df	SS	F	P>F
Year	3	7.86	0.04	0.9873
Diameter	1	8.81	7.24	0.0073**
Exp	1	2.15	0.14	0.7417
Site[Exp]	2	. 30.02	12.33	0.0001***
Exp*Year	3	18.80	0.42	0.7611
Complete vs. incomplete	1	6.11	5.02	0.0252*
Measurer[Yr]	8	487.43	50.05	0.0***
Cell order	1	0.01	0.01	0.9267
Cells per nest	1	112.34	92.28	0.0001***
Date	1	0.99	0.81	0.3681
Leaves per cell	1	16.83	13.82	0.0002**
Bore Depth	1	10.42	8.56	0.0035**
Bore Depth*Year	3	8.58	0.05	0.9855
Diameter*Bore depth	1	11.76	9.66	0.0019**
Diameter*Year* Bore Depth	3	9.70	0.05	0.9827
Diameter*Year	3	8.69	0.05	0.9853
Diameter* Leaves per cell	1	14.17	11.64	0.0007**
Model	35	949.53	22.29	0.0***
Error	1061	1291.62		

$\bar{X}$ = 15.6mm   CV = 7.1 r^2 = 0.42

TABLE 19: GLM of cell lengths for 1985 - 1988 M. inermis nests; female offspring and all diameters included.

CELL LENGTHS

Source of variation	df	SS	F	P>F
Year	3	5.54	0.19	0.9010
Diameter	1	0.00	0.00	0.9738
Exp	1	0.39	1.01	0.4214
Site[Exp]	2	0.77	0.40	0.6677
Exp*Year	3	5.33	4.59	0.1839
Complete vs. incomplete	1	0.12	0.13	0.7200
Measurer[Yr]	8	78.28	10.25	0.0001***
Cell order	1	1.57	1.65	0.2008
Cells per nest	1	8.21	8.60	0.0037**
Date	1	0.09	0.10	0.7549
Leaves per cell	1	1.50	1.57	0.2113
Bore Depth	1	0.03	0.03	0.8687
Bore Depth*Year	3	6.61	0.23	0.8762
Diameter*Bore depth	1	0.01	0.01	0.9261
Diameter*Year* Bore Depth	3	6.84	0.23	0.8710
Diameter*Year	3	5.97	0.20	0.8911
Diameter* Leaves per ell	1	1.46	1.53	0.2175
Model	35	218.12	6.53	0.0001***
Error	212	202.43		

$\bar{X}$ = 16.8mm   CV = 5.8 r^2 = 0.52

TABLE 20: Differences between observers in mean cell lengths (M.inermis, bore diameters greater than 9.5 only).

Measurer	Mean Cell Lengths m m	No. Cells Measured
LS (1985)	14.95	456
MS (1985)	15.34	197
JZ (1986)	15.83	69
KS (1986)	16.27	90
LS (1986)	14.22	59
MS (1986)	15.43	51
KS (1987)	16.62	377
LS (1987)	15.31	158
VS (1987)	15.32	321
BZ (1988)	15.62	144
KS (1988)	17.25	243
VS (1988)	15.77	423

Table 21: Sex Ratio by Site and Year for <u>M</u>. inermis.

| | 1985 | | | 1986 | | |
Site	Males	Females	Ratio	Males	Females	Ratio
C5	27	7	3.9	35	10	3.5
CL	25	12	2.1	6	2	3.0
F1	322	22	14.6	80	17	4.7
F2	141	37	3.8	177	29	6.1
Total	515	78	6.6	298	58	5.1

| | 1987 | | | 1988 | | |
Site	Males	Females	Ratio	Males	Females	Ratio
C5	104	36	2.9	46	27	1.7
CL	46	29	1.6	8	1	8.0
F1	315	141	2.2	132	27	4.9
F2	296	79	3.7	262	66	4.0
Total	761	285	2.7	448	121	3.7

TABLE 22: <u>M</u>. <u>relativa</u> Weights by Sex, Site and Year.

$$\overline{mg} \pm S.D.$$
$$(N)$$

Female

YEAR	C5	CL	F1	F2	TOTAL
Dry Weights					
1986	14.6 ± 2.6 (8)	13.5 ± 1.5 (3)	14.9 ± 4.7 (11)	14.8 ± 3.5 (18)	14.7 ± 3.5 (40)
1987	15.2 ± 2.2 (26)	14.1 ± 2.3 (11)	15.7 ± 1.5 (36)	15.8 ± 1.5 (6)	15.4 ± 1.9 (79)
1988	12.4 ± 2.8 (6)	10.6 ± 0.8 (3)	10.8 ± 3.1 (7)	13.3 ± 3.6 (5)	11.8 ± 2.9 (21)
Live Weights					
1987	41.5 ± 5.8 (18)	44.6 ± 5.1 (6)	43.6 ± 5.3 (19)	45.4 ± 3.7 (4)	43.1 ± 5.4 (47)
1988	39.8 ± 6.3 (23)	33.8 ± 3.2 (7)	31.5 ± 10.6 (8)	38.9 ± 8.3 (9)	37.3 ± 7.8 (47)

TABLE 22 continued

$$\overline{mg} \pm S.D.$$
(N)

Male

YEAR	C5	CL	F1	F2	TOTAL
Dry Weights					
1986	10.9 ± 2.5 (34)	10.2 ± 2.3 (28)	10.8 ± 3.2 (27)	11.9 ± 2.5 (86)	11.3 ± 2.6 (175)
1987	12.3 ± 2.3 (89)	11.4 ± 2.3 (35)	12.2 ± 2.0 (95)	11.5 ± 2.5 (26)	12.1 ± 2.2 (245)
1988	8.6 ± 1.7 (51)	9.2 ± 1.9 (12)	9.6 ± 1.9 (63)	8.5 ± 1.4 (20)	9.1 ± 1.8 (146)
Live Weights					
1987	35.5 ± 5.2 (73)	32.6 ± 5.7 (25)	35.5 ± 4.5 (112)	33.2 ± 6.4 (33)	34.9 ± 5.2 (243)
1988	27.7 ± 4.6 (63)	27.7 ± 4.8 (22)	31.0 ± 5.8 (106)	28.5 ± 4.4 (29)	29.4 ± 5.4 (220)

TABLE 23: <u>M</u>. <u>inermis</u> Weights by Sex, Site and Year.

$$\overline{mg} \pm S.D.$$
(N)

			Female		
YEAR	C5	CL	F1	F2	TOTAL
Dry Weights					
1986	46.0 ± 6.0 (4)	64.0 ± 18.8 (5)	58.5 (1)	---*	56.3 ± 15.8 (10)
1987	49.3 ± 19.3 (12)	50.1 ± 10.9 (6)	57.3 ± 9.9 (51)	54.2 ± 12.4 (21)	55.0 ± 12.3 (90)
1988	51.7 ± 3.8 (4)	---*	51.9 ± 5.0 (5)	44.8 ± 9.5 (18)	47.1 ± 8.7 (27)
Live Weights					
1987	---*	---*	181.7 ± 16.1 (9)	159.4 ± 38.8 (8)	171.2 ± 30.3 (17)
1988	149.4 ± 22.4 (24)	159.4 (1)	150.2 ± 22.1 (23)	146.8 ± 27.0 (63)	148.2 ± 24.8 (111)

* No bees weighed.

TABLE 23 continued.

$$\overline{mg} \pm \text{S.D.}$$
(N)

Male

YEAR	C5	CL	F1	F2	TOTAL
Dry Weights					
1986	29.0 ± 5.4 (4)	---*	37.2 ± 10.2 (29)	39.6 ± 7.7 (47)	38.2 ± 8.8 (80)
1987	37.7 ± 7.4 (29)	37.5 ± 6.0 (15)	38.4 ± 6.9 (144)	37.6 ± 6.6 (79)	38.0 + 6.8 (297)
1988	31.0 ± 5.6 (20)	31.0 ± 3.7 (6)	31.6 ± 6.7 (74)	32.6 ± 6.3 (143)	32.1 ± 6.3 (243)
Live Weights					
1987	100.6 ± 22.8 (7)	---*	111.7 ± 19.1 (39)	112.1 ± 22.6 (46)	111.1 ± 21.2 (92)
1988	97.1 ± 16.9 (43)	95.8 ± 11.7 (8)	98.5 ± 18.0 (118)	103.7 ± 20.3 (227)	101.3 ± 19.3 (396)

TABLE 24: Mean number of cells per <u>Megachile</u> <u>relativa</u> nest.

MEAN NUMBER OF CELLS PER NEST

$\bar{X}$ ± standard deviation
(N)

	C5	CL	F1	F2
1985	4.15 ±2.56 (26)	4.33 ±2.91 (33)	6.46 ±2.74 (37)	3.72 ±1.81 (57)
1986	4.00 ±2.00 (31)	3.72 ±2.19 (36)	5.06 ±2.85 (31)	5.12 ±2.65 (50)
1987	6.17 ±2.57 (60)	4.05 ±2.35 (40)	5.72 ±2.68 (60)	2.94 ±1.56 (33)
1988	3.05 ±1.54 (65)	2.37 ±1.44 (41)	4.08 ±2.17 (50)	2.97 ±1.77 (32)

	CONTROL AREAS	EXPERIMENTAL AREAS
1985	4.25 ±2.74 (59)	4.80 ±2.59 (94)
1986	3.85 ±2.09 (67)	5.10 ±2.71 (81)
1987	5.32 ±2.68 (100)	4.73 ±2.69 (93)
1988	2.78 ±1.53 (106)	3.65 ±2.08 (82)

TABLE 25: Categorical modeling of number of cells per complete nest of _Megachile relativa_, 1985-1988.

NUMBER OF CELLS PER COMPLETE NEST

Source of variation	df	Chi.Square	Prob.
Intercept	3	34.03	0.0001***
Exp	3	8.80	0.0321*
Site[Exp]	6	50.52	0.0001***
Year	9	45.67	0.0001***
Exp*Year	9	22.03	0.0088**
Likelihood Ratio	18	45.26	0.0004***

TABLE 26: Mean number of cells per <u>Megachile</u> <u>inermis</u> nest.

MEAN NUMBER OF CELLS PER NEST

$\overline{X}$ ± standard deviation
(N)

	C5	CL	F1	F2
1987	4.82 ±1.62 (22)	4.54 ±1.45 (13)	5.56 ±1.57 (59)	5.62 ±1.40 (52)
1988	4.27 ±1.25 (26)	4.00 ±1.63 (4)	5.03 ±1.55 (38)	4.58 ±1.48 (119)

	CONTROL AREAS	EXPERIMENTAL AREAS
1987	4.71 ±1.54 (35)	5.59 ±1.49 (111)
1988	4.23 ±1.28 (30)	4.69 ±1.51 (157)

TABLE 27: Categorical modeling of number of cells per complete nest of Megachile inermis, 1987-1988 (diameters > 9.5mm, bore depths > 135mm).

NUMBER OF CELLS PER COMPLETE NEST

Source of variation	df	Chi.Square	Prob.
Intercept	1	15.19	0.0001***
Exp	1	9.51	0.0020**
Site[Exp]	2	0.62	0.7344
Year	1	3.17	0.0752
Exp*Year	1	0.05	0.8244
Likelihood Ratio	2	3.94	0.1393

TABLE 28: GLM of LOG leaves per cell in 1985 - 1988 M. inermis nests with bore diameters of 9.5mm or larger.

LEAVES PER CELL

Source of variation	df	SS	F	P>F
Year	3	0.23	1.72	0.1609
Diameter	1	0.04	0.94	0.3320
Exp	1	0.84	12.80	0.0700
Site[Exp]	2	0.13	1.50	0.2237
Exp*Year	3	0.52	2.67	0.2845
Complete vs. incomplete	1	1.12	25.65	0.0001***
Cell order	1	3.21	73.34	0.0001***
Cells per nest	1	0.04	1.02	0.3115
Date	1	8.97	204.83	0.0***
Diameter*Year	3	0.25	1.92	0.1248
Bore depth	1	0.05	1.11	0.2927
Bore depth*Year	3	0.20	1.52	0.2066
Diameter*Bore depth	1	0.05	1.07	0.3003
Diameter*Year* Bore depth	3	0.22	1.70	0.1649
Model	25	41.06	37.53	0.0***
Error	2570	112.49		

$\bar{X}$ = 2.47 (11.8 leaves)   CV = 8.5 r^2 = 0.27

TABLE 29: GLM of LOG leaves per cell from cells whose offspring sex is known in 1985 - 1988 M. inermis nests, all bore diameters.

LEAVES PER CELL

Source of variation	df	SS	F	P>F
Year	3	0.34	2.75	0.0416*
Diameter	1	0.00	0.00	0.9778
Exp	1	0.95	11.54	0.0768
Site[Exp]	2	0.16	2.02	0.1334
Exp*Year	3	0.84	3.42	0.2347
Complete vs. incomplete	1	0.11	2.79	0.0948
Cell order	1	0.35	8.54	0.0035**
Cells per nest	1	0.11	2.66	0.1030
Date	1	4.33	106.07	0.0001***
Diameter*Year	3	0.30	2.44	0.0628
Bore depth	1	0.00	0.00	0.9437
Bore depth*Year	3	0.34	2.77	0.0406*
Diameter*Bore depth	1	0.00	0.00	0.9702
Diameter*Year* Bore depth	3	0.30	2.48	0.0593
Sex	1	1.60	39.15	0.0001***
Sex*Year	3	0.20	1.67	0.1715
Model	29	34.12	28.81	0.0***
Error	1319	53.87		

$\bar{X} = 2.43$ (11.4 leaves)   CV = 8.3 $r^2 = 0.39$

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TABLE 30: GLM of LOG leaves per cell from cells with male offspring, 1985 - 1988 M. inermis nests (all diameters).

LEAVES PER CELL

Source of variation	df	SS	F	P>F
Year	3	0.30	2.35	0.0710
Diameter	1	0.01	0.28	0.5961
Exp	1	1.23	18.48	0.0501
Site[Exp]	2	0.13	1.57	0.2076
Exp*Year	3	0.86	4.27	0.1954
Complete vs. incomplete	1	0.18	4.29	0.0386*
Cell order	1	0.63	14.74	0.0001***
Cells per nest	1	0.01	0.24	0.6228
Date	1	3.58	84.33	0.0001***
Diameter*Year	3	0.32	2.51	0.0572
Bore depth	1	0.01	0.28	0.5991
Bore depth*Year	3	0.33	2.58	0.0525
Diameter*Bore depth	1	0.02	0.41	0.5205
Diameter*Year* Bore depth	3	0.35	2.73	0.0426*
Model	25	30.27	28.55	0.0***
Error	1075	45.59		

$\bar{X}$ = 2.46 (11.7 leaves)   CV = 8.4 r^2 = 0.40

TABLE 31: GLM of LOG leaves per cell from cells with female offspring, 1985 - 1988 M. inermis nests (all diameters).

LEAVES PER CELL

Source of variation	df	SS	F	P>F
Year	3	0.18	2.03	0.1103
Diameter	1	0.01	0.28	0.5986
Exp	1	0.00	0.00	0.9646
Site[Exp]	2	0.10	1.67	0.1909
Exp*Year	3	0.44	2.91	0.2663
Complete vs. incomplete	1	0.00	0.04	0.8500
Cell order	1	0.08	2.59	0.1088
Cells per nest	1	0.10	3.31	0.0704
Date	1	0.55	18.38	0.0001***
Diameter*Year	3	0.18	2.03	0.1101
Bore depth	1	0.00	0.16	0.6921
Bore depth*Year	3	0.19	2.09	0.1029
Diameter*Bore depth	1	0.01	0.26	0.6124
Diameter*Year* Bore depth	3	0.18	2.05	0.1085
Model	25	3.49	4.64	0.0001***
Error	222	6.68		

$\bar{X}$ = 2.36 (10.6 leaves)   CV = 7.4 r^2 = 0.34

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TABLE 32: CHI-SQUARE HETEROGENEITY GOODNESS OF FIT ANALYSIS

H_0: Nest orientations at each hutch set are homogeneous between years in a 50% E-W, 50% N-S distribution, so that data for a hutch set can be pooled across years.

	EW	NS	Total	χ^2	df			EW	NS	Total	χ^2	df	
		C5-S[1]							CL-E[1]				
1985	6	5	11	0.091	1	n.s.		15	9	24	1.500	1	n.s.
1986	4	6	10	0.400	1	n. s.		8	3	11	2.273	1	n.s.
1987	6	16	22	4.545	1	.025 <P<.05		12	6	18	2.000	1	n.s.
1988	4	19	23	9.783	1	.001<P<.005		13	2	15	8.067	1	.001<P<.005
		C5-N[3]							CL-N[1]				
1985	4	2	6	---				12	7	19	1.316	1	n.s.
1986	5	6	11	0.091	1	n.s.		10	7	17	0.529	1	n.s.
1987	4	3	7	---				10	3	13	3.769	1	n.s.
1988	12	3	15	5.400	1	.01<P<.025		17	4	21	8.048	1	.001<P<.005
		C5-W[2]							CL-W[3]				
1985	8	14	22	1.636	1	n.s.		7	4	11	0.818	1	n.s.
1986	11	7	18	0.889	1	n.s.		6.	8	14	0.286	1	n.s.
1987	18	18	36	0.000	1	n.s.		5	6	11	0.091	1	n.s.
1988	14	24	38	2.632	1	n.s.		3	6	9	---		

C5-W
Totals   51   63 | 114   1.263 1 n.s.

Total of X2s   5.157 4

χ^2s of totals   1.263 1
                        3.894 3 n.s.

χ^2 of totals with Yates correction: 1.061 1 n.s.

[1] Data for these hutch sets are heterogeneous between years.

[2] Data for this hutch sets is homogeneous across years. The Yates correction is not applied until the final heterogeneity χ^2 (Zar, 1974, p. 51)

[3] Sample sizes are too small to apply a Chi-Square test to data for some years, so heterogeneity test can't be made.

OF <u>M</u>. <u>RELATIVA</u> NEST ORIENTATION BY HUTCH SET AND YEAR

H_1: Nest orientations at each hutch set are not in a 50% E-W, 50% N-S distribution and/or are heterogeneous, so data cannot be pooled across years.*

EW	NS	Total	χ^2	df			EW	NS	Total	χ^2	df	
		F1-E[1]							F2-E[1]			
15	6	21	3.857	1	.025<P<.05		9	5	14	1.143	1	n.s.
12	4	16	4.000	1	.025<P<.05		10	16	26	1.385	1	n.s.
18	21	39	0.231	1	n.s.		6	9	15	0.600	1	n.s.
7	9	16	0.250	1	n.s.		4	**16**	20	7.200	1	.005<P<.01
		F1-N[1]							F1-N[1]			
15	5	20	5.000	1	n.s.		20	17	37	0.243	1	n.s.
5	8	13	1.646	1	n.s.		10	**23**	33	5.313	1	.01<P<.025
6	**16**	22	4.545	1	.025<P<.05		7	10	17	0.529	1	n.s.
4	**22**	26	12.462	1	P<.001		5	8	13	0.692	1	n.s.
		F1-W[3]							F1-W[3]			
2	**12**	14	7.143	1	.01<P<.005		8	10	18	0.222	1	n.s.
4	2	6	---				5	1	6	---		
2	2	4	---				2	4	6	---		
10	5	15	1.667	1	n.s.		3	3	6	---		

* #'s in bold indicate a directional preference.

TABLE 33: LOG-LIKELIHOOD RATIO HETEROGENEITY CONTINGENCY
HUTCH SET AND YEAR

H_0: Nest orientations at each hutch set are homogeneous between years (i.e.,
have the same directional preference)*

	EW	NS	R			EW	NS	R	
		C5-S					CL-E		
1985	6	5	11			15	9	24	
1986	4	6	10	G=5.3161		8	3	11	G=3.0477
1987	6	16	22	df = 3		12	6	18	df=3
1988	4	19	23	n.s.		13	2	15	n.s.
C	20	46	66			48	20	68	
		C5-N					CL-N		
1985	4	2	6			12	7	19	
1986	5	6	11	G=3.5513		10	7	17	G=2.9824
1987	4	3	7	df=3		10	3	13	df=3
1988	12	3	15	n.s.		17	4	21	n.s.
C	25	14	39			49	21	70	
		C5-W					CL-W		
1985	8	14	22			7	4	11	
1986	11	7	18	G=3.9510		6	8	14	
1987	18	18	36	df=3		5	6	11	G=2.256
1988	14	24	38	n.s.		3	6	9	df=3
C	51	63	114			21	24	45	n.s.

	C5 - BY HUTCH SETS				CL - BY HUTCH SETS				
C5-S	20	46	66		CL-E	48	20	68	
C5-N	25	14	39	G=23.8780[1]	CL-N	49	21	70	G=8.027[1]
C5-W	51	63	114	df=2	CL-W	21	24	45	df=3
C	96	134	230	P<.001		118	65	183	P<.05

* #'s in bold indicate a preference in the direction of that number for
the hutch set.

1. Within hutch sets, data are homogeneous between years. However, hutch sets (data
pooled across years) are heterogeneous. Thus, hutch set data cannot be pooled by year.
2. Within hutch sets data are heterogeneous; cannot be pooled.
3. Data is homogeneous within hutch sets across years and between hutch sets. Data pooled
by year across hutch sets is also homogeneous, with a preference toward N-S nest entrances.

TABLES FOR $\underline{M}$. RELATIVA NEST ENTRANCE ORIENTATION BY

H_1: Nest orientations at each hutch set are heterogeneous between years and hutch sets at a site, so data cannot be pooled.

EW	NS	R		EW	NS	R	
	F1-E				F2-E		
15	6	21		9	5	14	
12	4	16	$G=7.0828^2$	10	16	26	$G=6.9829$
18	21	39	df=3	6	9	15	df=3
7	9	16	n.s.	4	16	20	n.s.
52	40	92		29	46	75	
	F1-N				F2-N		
15	5	20		20	17	37	
5	8	13	$G=9.4297^2$	10	23	33	$G=4.1659$
6	16	22	df=3	7	10	17	df=3
4	22	26	P<.025	5	8	13	n.s.
30	51	81		42	58	100	
	F1-W				F2-W		
2	12	14		8	10	18	
4	2	6	$G=10.0725^2$	5	1	6	$G=3.8133$
2	2	4	df=3	2	4	6	df=3
10	5	15	P<0.25	3	3	6	n.s.
18	21	9		18	18	36	

F2 BY HUTCH SET

	EW	NS	R	
F2- E	29	46	75	$G=1.2757^3$
F-2 N	42	58	100	df=2
F2- W	18	18	36	n.s.
	89	122	211	

POOLED DATA BY YR.

	EW	NS	R	
1985	37	32	69	
1986	25	40	65	$G=6.2907^3$
1987	15	23	38	df=3
1988	12	27	39	n.s.
	89	122	211	

Total of G tests 1-3	14.9621
G-test of totals	- 6.2907
Heterogeneity G	8.6714
df = 9 - 3 = 6	n.s.

TABLE 34: Number of individual bees of <u>M</u>. <u>inermis</u> and number of LO trip durations timed by each observer at each site, 1983-1986.

| Observers | Control Sites | | Test Sites | | |
	Camp 5	County Line	Ford 1 (north)	Ford 2 (south)	Totals
Number of bees timed:					
1983					
AP			1	4	5
KG			6		6
1984					
JH		2	4	3	9
KG			1		1
VS			5		5
1986					
PW	2		7	6	15
Totals	2	2	24	13	41
Numbers of LO trips timed:					
1983					
AP			10	26	36
KG			43		43
1984					
JH		13	46	27	86
KG			9		9
VS			55		55
1986					
PW	26		38	55	119
Totals	26	13	201	108	348
Average no. trips per bee:					
	13.0	6.5	8.4	8.3	8.5

TABLE 35: Number of cell caps of <u>M. inermis</u> and number of LO trip durations timed by each observer at each site, 1987.

Observers	Control Sites		Test Sites		Totals
	Camp 5	County Line	Ford 1 (north)	Ford 2 (south)	
Number of cell caps timed:					
FB	8		5	3	16
JZ	9	3	7	3	22
KR	13	2	12	7	34
KS	2	2	1		5
SM	5	3	12	11	31
Totals	37	10	37	24	108
Numbers of LO trips timed:					
FB	34		21	11	66
JZ	46	22	27	20	115
KR	102	13	88	34	237
KS	14	9	4		27
SM	21	31	66	66	184
Totals	217	75	206	131	630
Average no. trips per cell cap:					
	5.9	7.5	5.6	5.4	5.8

TABLE 36: Number of cell caps of M. inermis and number of LO trip durations timed by each observer at each site, 1988.

| Observers | Control Sites | | Test Sites | | Totals |
	Camp 5	County Line	Ford 1 (north)	Ford 2 (south)	
Number of cell caps timed:					
DS	7	1	8	10	26
RL	5	1	6	7	19
SO	2	1	4	5	12
TC	7	2	7	8	24
VS	5	1	5	5	16
Totals	26	6	30	35	97
Numbers of LO trips timed:					
DS	37	2	40	49	128
RL	23	14	32	42	111
SO	12	5	30	36	83
TC	37	10	39	38	124
VS	31	6	30	25	92
Totals	140	37	171	190	538
Average no. trips per cell cap:					
	5.4	6.2	5.7	5.4	5.5

TABLE 37: Number of cell caps of M. inermis and number of LO trip durations timed by each observer at each site, 1987. Trip numbers 1-8 only.

| Observers | Control Sites | | Test Sites | | Totals |
	Camp 5	County Line	Ford 1 (north)	Ford 2 (south)	
Number of cell caps timed:					
FB	4		2	2	8
JZ	4	2	3	1	10
KR	6		4	1	11
KS			1		1
SM	2		5	4	11
Totals	16	2	15	8	41
Numbers of LO trips timed:					
FB	17		7	3	27
JZ	17	8	9	8	42
KR	37		18	3	58
KS			1		1
SM	6		21	19	46
Totals	77	8	56	33	174
Average no. trips per cell cap:					
	4.8	4.0	3.7	4.1	4.2

TABLE 38: Number of cell caps of <u>M</u>. <u>inermis</u> and number of LO trip durations timed by each observer at each site, 1988. Trip numbers 1-8 only.

| Observers | Control Sites | | Test Sites | | Totals |
	Camp 5	County Line	Ford 1 (north)	Ford 2 (south)	
Number of cell caps timed:					
DS	6		6	7	19
RL	4	1	6	6	17
SO	1		3	5	9
TC	5	2	3	3	13
VS	4	1	5	5	15
Totals	20	4	23	26	73
Numbers of LO trips timed:					
DS	32		33	38	103
RL	18	8	32	35	93
SO	6		16	31	53
TC	29	8	17	13	67
VS	25	6	30	25	86
Totals	110	22	128	142	402
Average no. trips per cell cap:					
	5.0	5.5	5.6	5.5	5.5

TABLE 39: Number of cell caps of <u>M</u>. <u>inermis</u> and number of LO trip durations timed by each observer at each site, 1989. Trip numbers 1-8 only.

Observers	Control Sites		Test Sites		Totals
	Camp 5	County Line	Ford 1 (north)	Ford 2 (south)	
Number of cell caps timed:					
CD	9	5	11	11	36
MP	10	6	9	8	33
TC	9	5	10	10	34
TT	10	4	10	10	34
Totals	38	20	40	39	137
Numbers of LO trips timed:					
CD	49	26	55	53	183
MP	60	35	53	50	198
TC	49	25	50	51	175
TT	53	22	53	53	181
Totals	211	108	211	207	737
Average no. trips per cell cap:					
	5.6	5.4	5.3	5.3	5.4

TABLE 40: GLM of log log transformed LO trip durations for M. inermis, 1989; trips 1-8 only.

LO TRIP DURATIONS

Source of variation	df	SS	F	P>F
Exp	1	0.24	1.26	0.3776
Site[Exp]	2	0.37	4.79	0.0085**
Trip number	1	3.30	84.50	0.0001***
Observer	3	0.16	1.36	0.2552
Time of day	1	0.23	5.89	0.0155*
Time*Time	1	0.18	4.68	0.0308*
Date	1	0.02	0.41	0.5238
Model	10	4.7	11.98	0.0001***
Error	726	28.34		
$\bar{X}$ = 1.1 (20.7 sec.)	CV = 17.8	r^2 = 0.14		

TABLE 41: GLM of log log transformed LO trip durations for M. inermis, 1987-89. Only trips 1-8 for which trip number was certain are included in this analysis.

LO TRIP DURATIONS

Source of variation	df	SS	F	P>F
Year	2	0.16	1.00	0.4002
Exp	1	0.77	13.83	0.0653
Site[Exp]	2	0.11	1.47	0.2311
Exp*Year	2	0.10	0.91	0.5243
Trip number	1	4.53	120.17	0.0001***
Trip number*Year	2	0.06	0.41	0.6739
Observer[Year]	11	0.87	2.09	0.0187*
Time of day	1	0.08	2.12	0.1461
Time*Time	1	0.04	1.14	0.2864
Date	1	0.01	0.30	0.5820
Model	24	11.70	12.92	0.0***
Error	1288	48.59		

$\bar{X} = 1.1$ (22.3 sec.)   CV = 17.1 $r^2 = 0.19$

TABLE 42: GLM of log transformed LO trip durations for M. inermis, 1983 - 1986.

LO TRIP DURATIONS

Source of variation	df	SS	F	P>F
Exp	1	1.1	0.82	0.4615
Site[Exp]	2	2.7	2.44	0.0892
Trip rank	1	25.5	46.73	0.0001***
Measurer	3	7.1	4.31	0.0054**
Time of day	1	0.0	0.18	0.6704
Time*Time	1	0.0	0.02	0.8828
Date	1	15.3	28.22	0.0001***
Model	10	60.1	11.04	0.0***
Error	301	163.9		
$\bar{X}$ = 3.6 (31 sec.)	CV = 20.3	r^2 = 0.27		

TABLE 43: Late Summer Emergences (% bivoltinism) of M. relativa and Coelioxys sp.

M. relativa

Year	cells emerging late summer / total cells emerging[1]	(%)	nests emerging late summer / total nests emerging[1]	(%)
1987	33/629	(5.2%)	7/186	(3.8%)
1988	13/285	(4.6%)	7/144	(4.9%)
1989	81/792[3]	(10.2%)	19/198[3]	(9.6%)

Coelioxys sp.

Year	cells emerging late summer / total cells emerging[2]	(%)	nests emerging late summer / total nests emerging[2]	(%)
1987	11/99	(11.1%)	10/77	(13.0%)
1988	10/87	(11.5%)	8/62	(12.9%)
1989	15/?		9/?	

[1]Total cells or nests with adult M. relativa.
[2]Total cells or nests with adult Coelioxys in M. relativa nests.
[3]Estimate, since spring emergence has not yet taken place.

TABLE 44: Late Summer Emergences (% bivoltinism) of M. inermis and Coelioxys sp.

<u>M. inermis</u>

Year	cells emerging late summer / total cells emerging[1]	(%)	nests emerging late summer / total nests emerging[1]	(%)
1987	2/1,011	(0.2%)	1/262	(0.4%)
1988	0/563	(0.0%)	0/168	(0.0%)
1989	3/2016[3]	(0.1%)	1/504[3]	(0.2%)

<u>Coelioxys</u> sp.

Year	cells emerging late summer / total cells emerging[2]	(%)	nests emerging late summer / total nests emerging[2]	(%)
1987	0/62	(0.0%)	0/48	(0.0%)
1988	0/18	(0.0%)	0/16	(0.0%)
1989	0/?	(0.0%)	0/?	(0.0%)

[1]Total cells or nests with adult M. inermis.
[2]Total cells or nests with adult Coelioxys in M. inermis nests.
[3]Estimate, since spring emergence has not yet taken place.

TABLE 45: Proportion of M. relativa mortality from various sources by site.

Stage or source of mortality	SITE			
	C5	CL	F1	F2
1985				
Pre-overwintering (egg & larvae)	0.187	0.131	0.059	0.053
Overwintering (Prepupae)	0.045	0.069	0.014	0.041
Total parasitism	0.090	0.073	0.100	0.254
(Coelioxys only)	(0.077)	(0.053)	(0.089)	(0.234)
Post-overwintering Survival*	0.677	0.727	0.827	0.652
1986				
Pre-overwintering (egg & larvae)	0.103	0.138	0.109	0.042
Overwintering (Prepupae)	0.129	0.015	0.085	0.064
Total parasitism	0.174	0.169	0.127	0.137
(Coelioxys only)	(0.135)	(0.131)	(0.127)	(0.102)
Post-overwintering Survival*	0.594	0.677	·0.679	0.757
1987				
Pre-overwintering (egg & larvae)	0.235	0.354	0.187	0.344
Overwintering (Prepupae)	0.041	0.030	0.055	0.070
Total parasitism	0.058	0.128	0.118	0.234
(Coelioxys only)	(0.041)	(0.122)	(0.111)	(0.195)
Post-overwintering Survival*	0.665	0.488	0.639	0.352
1988				
Pre-overwintering (egg & larvae)	0.313	0.407	0.363	0.464
Overwintering (Prepupae)	0.134	0.122	0.106	0.064
Total parasitism	0.167	0.228	0.099	0.144
(Coelioxys only)	(0.138)	(0.195)	(0.070)	(0.128)
Post-overwintering Survival*	0.386	0.244	0.433	0.328

* Includes cells with dead pupae, dead adults, and successfully emerging adult M. relativa.

TABLE 46: Proportion of M. inermis mortality from various sources by site.

Stage or source of mortality	SITE			
	C5	CL	F1	F2
1985				
Pre-overwintering (egg & larvae)	0.151	0.098	0.184	0.114
Overwintering (Prepupae)	0.019	0.000	0.029	0.022
Total parasitism	0.189	0.176	0.031	0.079
(Coelioxys only)	(0.170)	(0.059)	(0.011)	(0.035)
Post-overwintering Survival*	0.642	0.725	0.757	0.786
1986				
Pre-overwintering (egg & larvae)	0.123	0.000	0.061	0.004
Overwintering (Prepupae)	0.053	0.000	0.038	0.026
Total parasitism	0.035	0.000	0.167	0.074
(Coelioxys only)	(0.000)	(0.000)	(0.038)	(0.009)
Post-overwintering Survival*	0.789	1.000 ·	0.735	0.896
1987				
Pre-overwintering (egg & larvae)	0.272	0.062	0.131	0.124
Overwintering (Prepupae)	0.092	0.062	0.055	0.069
Total parasitism	0.072	0.186	0.070	0.101
(Coelioxys only)	(0.048)	(0.088)	(0.032)	(0.039)
Post-overwintering Survival*	0.564	0.690	0.744	0.705
1988				
Pre-overwintering (egg & larvae)	0.172	0.300	0.175	0.260
Overwintering (Prepupae)	0.164	0.100	0.092	0.086
Total parasitism	0.034	0.150	0.039	0.056
(Coelioxys only)	(0.009)	(0.150)	(0.000)	(0.022)
Post-overwintering Survival*	0.629	0.450	0.694	0.597

* Includes cells with dead pupae, dead adults, and successfully emerging adult M. inermis.

TABLE 47: ANOVA of arcsine transformed proportion of prepupal mortality for M. relativa, 1985 - 1988.

PROPORTION OF PREPUPAL MORTALITY

Source of variation	df	SS	F	P>F
Year	3	0.11	5.58	0.0360*
Exp	1	0.00	0.75	0.4786
Site[Exp]	2	0.01	0.41	0.6821
Exp*Year	3	0.02	2.59	0.2911
Model	9	0.13	2.34	0.1570
Error	6	0.04		
$\bar{X}$ = 0.30	CV = 26.2	r^2 = 0.78		

TABLE 48: Multiple comparison tests for differences between years in the proportion of prepupal mortality for <u>M</u>. <u>relativa</u> 1985-1988.

DUNCAN GROUPING	MEAN	N	YEAR	SNK GROUPING
A	0.43982	4	1988	A
B	0.28530	4	1986	B
B	0.27223	4	1987	B
B	0.22101	4	1985	B

$\alpha = 0.05$ df=6

TABLE 49: ANOVA of arcsine transformed proportion of prepupal mortality for M. inermis, 1985 - 1988.

PROPORTION OF PREPUPAL MORTALITY

Source of variation	df	SS	F	P>F
Year	3	0.14	94.12	0.0001***
Exp	1	0.00	0.37	0.6034
Site[Exp]	2	0.02	15.42	0.0043**
Exp*Year	3	0.01	0.47	0.7319
Model	9	0.17	37.88	0.0001***
Error	6	0.00		
$\bar{X} = 0.27$	CV = 8.3	$r^2 = 0.98$		

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TABLE 50: Multiple comparison tests for differences between years in the proportion of prepupal mortality for M. inermis 1985-1988.

DUNCAN GROUPING	MEAN	N	YEAR	SNK GROUPING
A	0.40022	4	1988	A
B	0.30410	4	1987	B
C	0.21036	4	1986	C
D	0.15555	4	1985	D

$\alpha = 0.05$ df=6

TABLE 51: ANOVA of arcsine transformed proportion of prepupal mortality for <u>M</u>. <u>relativa</u>, 1988 only.

PROPORTION OF PREPUPAL MORTALITY

Source of variation	df	SS	F	P>F
Exp	1	0.02	3.88	0.1878
Site[Exp]	2	0.01	1.09	0.4775
Direction	1	0.00	0.59	0.5220
Direction*Exp	1	0.02	3.68	0.1951
Model	5	0.05	2.21	0.3403
Error	2	0.01		
$\bar{X} = 0.46$	CV = 14.3	$r^2 = 0.85$		

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TABLE 52: ANOVA of arcsine transformed proportion of prepupal mortality for <u>M</u>. <u>inermis</u>, 1988 only.

PROPORTION OF PREPUPAL MORTALITY

Source of variation	df	SS	F	P>F
Exp	1	0.02	19.53	0.0476*
Site[Exp]	2	0.00	5.57	0.1522
Direction	1	0.00	8.09	0.1045
Direction*Exp	1	0.02	20.08	0.0464*
Model	5	0.05	47.96	0.0206*
Error	2	0.00		
$\bar{X} = 0.43$	CV = 3.3	$r^2 = 0.99$		

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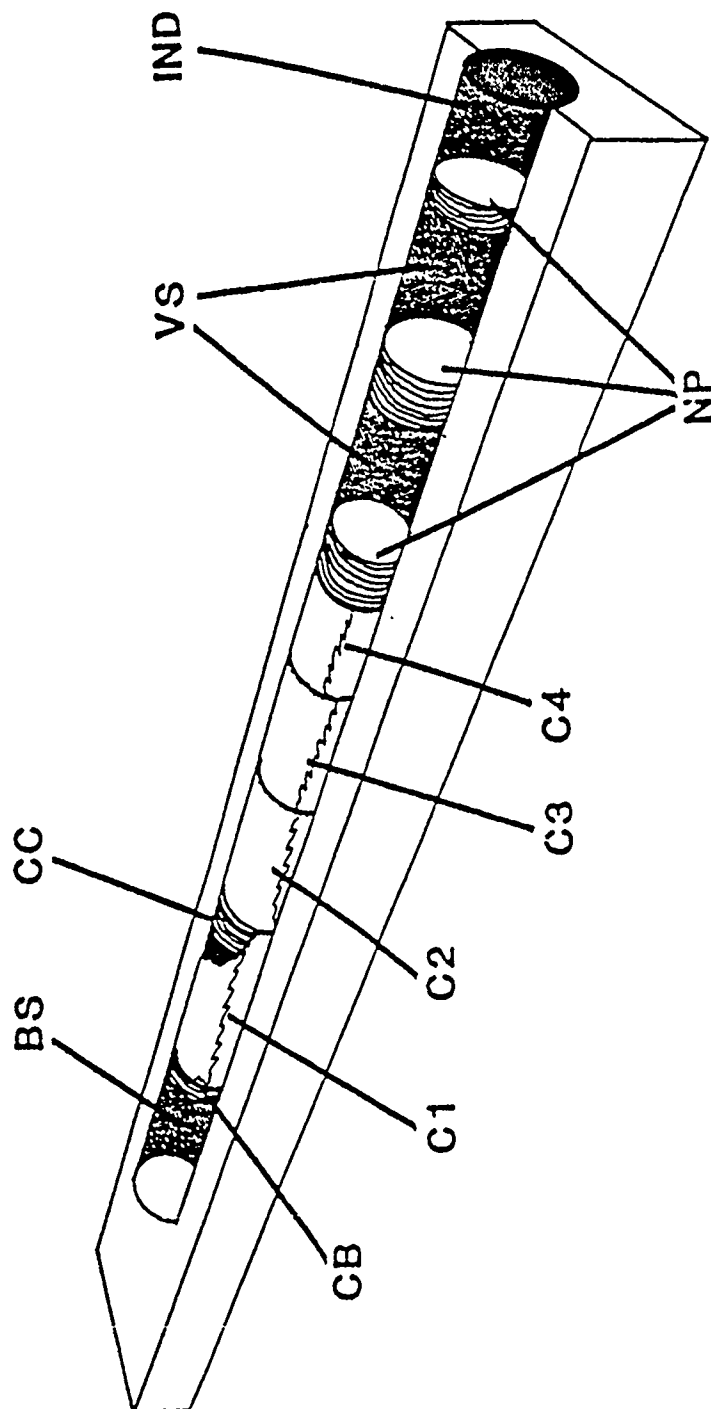
Figure 1. Cut away view of a completed Megachile nest.

BS - Basal Space; CB - Cell Base; C1, C2, C3, C4 - Reproductive Cells 1 through 4; CC - Cell Cap; NP - Nest Plugs; VS - Vestibular Spaces; IND - Indentation.

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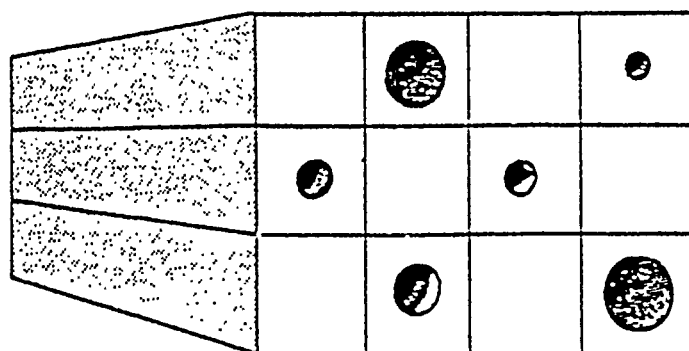
Figure 2. Example of arrangement of nests in block, 1983 - 1986.
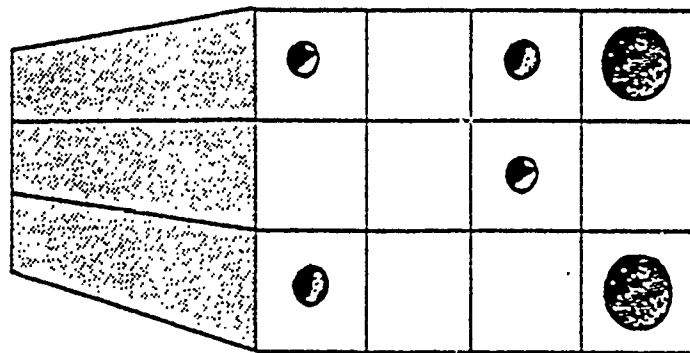
Figure 3. Example of arrangement of nests in block, 1987-1989.

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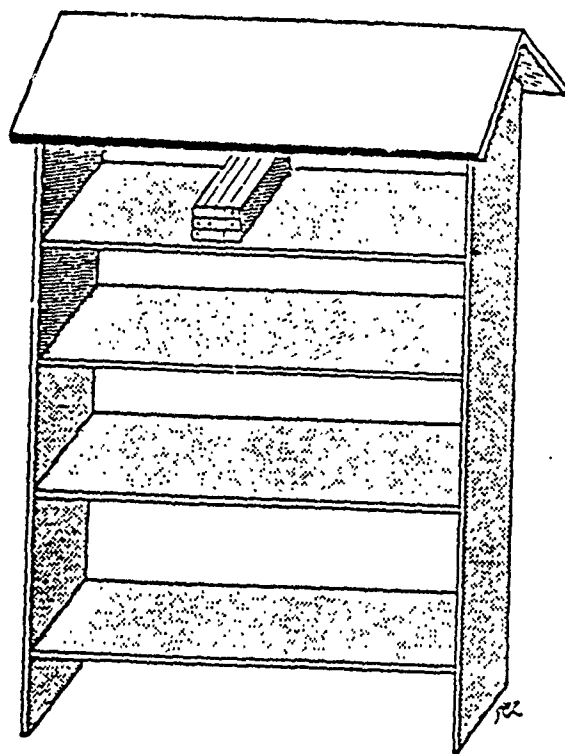
Figure 4. Hutch, with one block of nests.

↔ Cell Length Including Cap Length
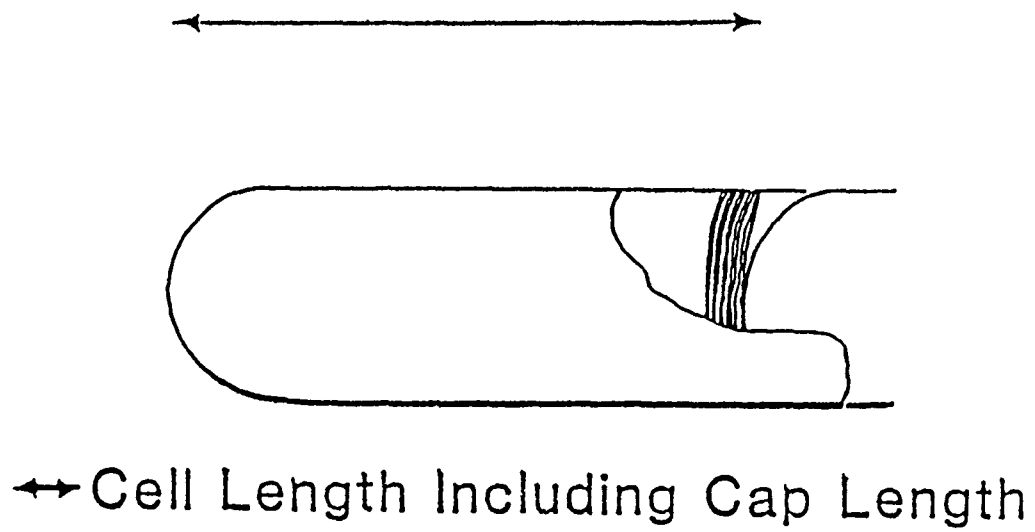
Figure 5.   A single reproductive cell, indicating how cell lengths are measured.

CUMULATIVE NUMBER OF NESTS OVER TIME
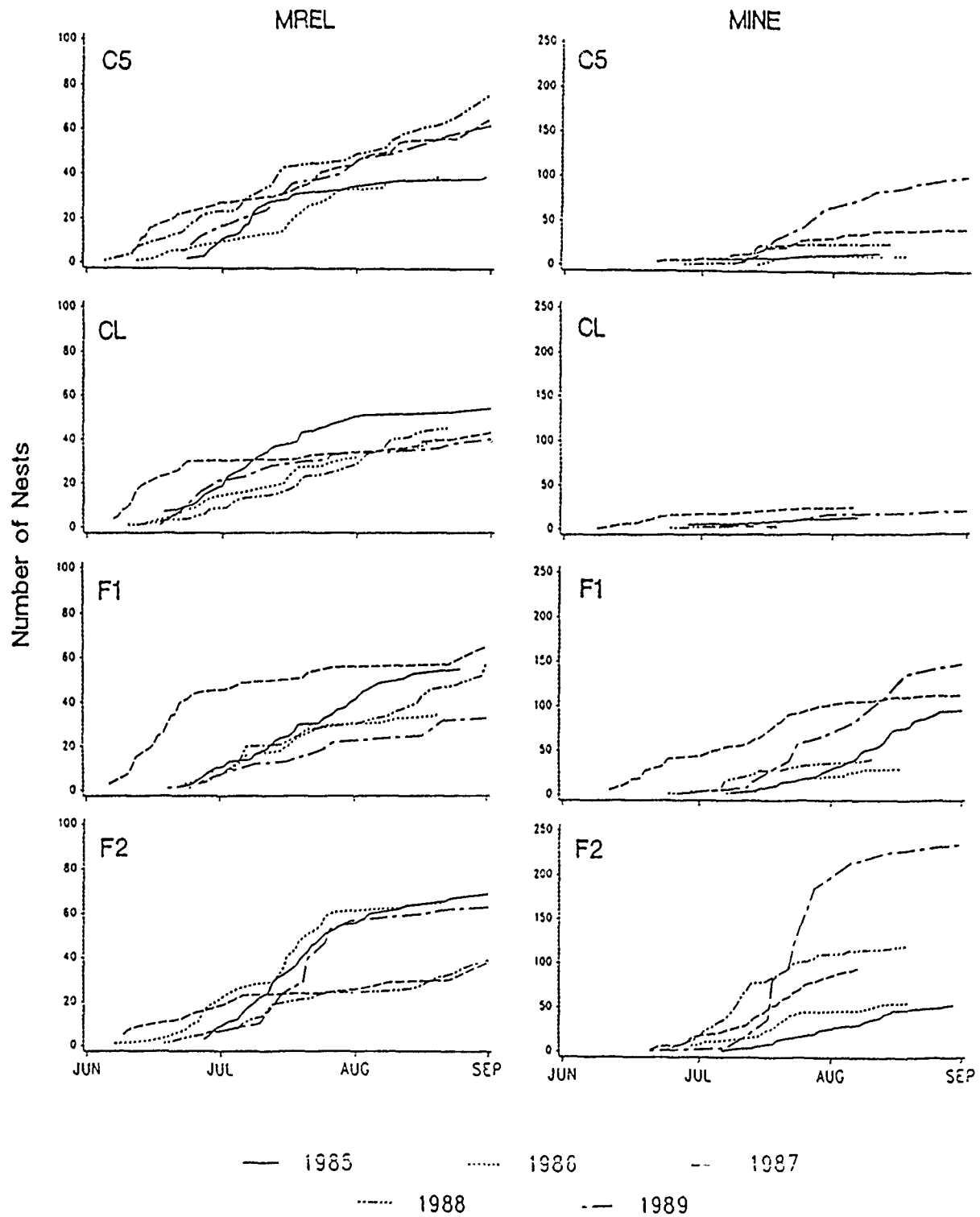
Figure 6. Cumulative number of nests of _M. relativa_ and _M. inermis_ at each site, 1985-1989. Note different scales for each species.
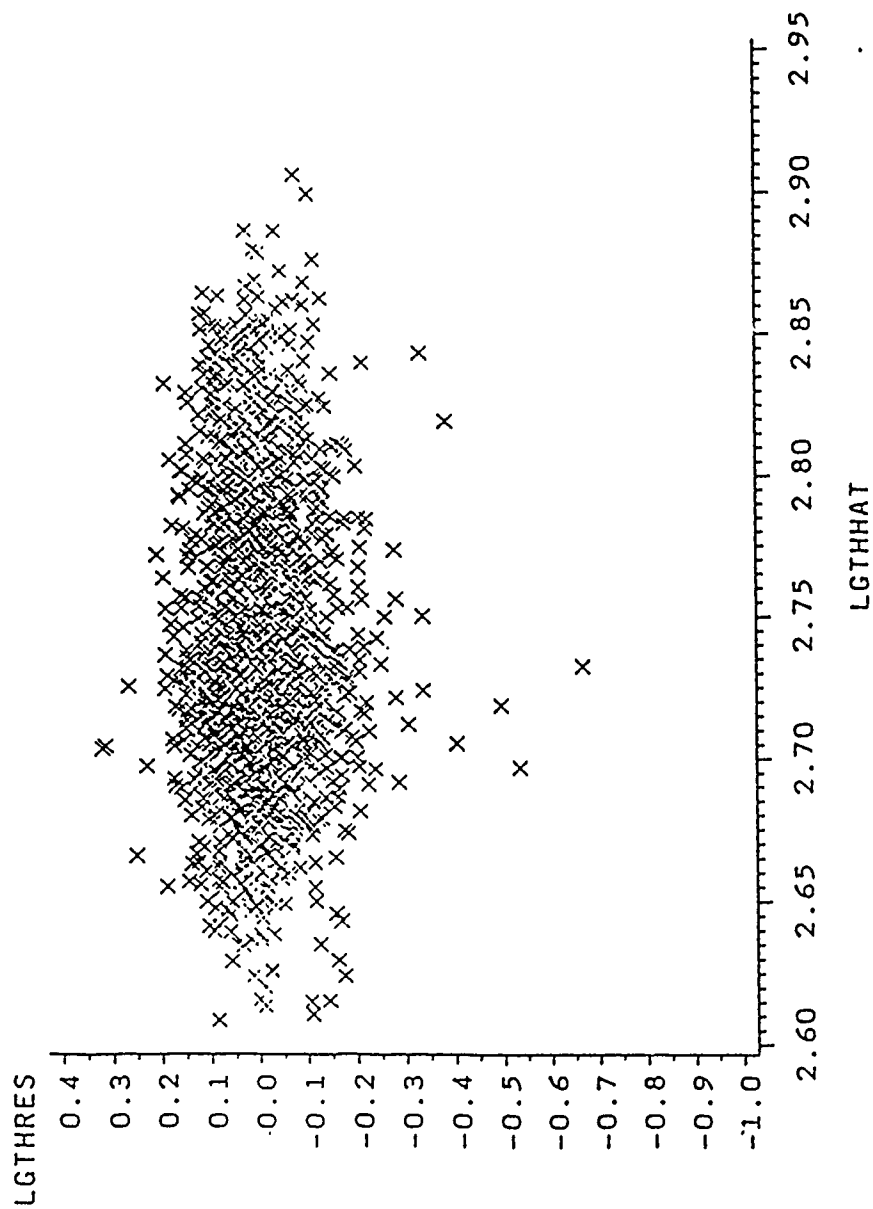
Figure 7. Plot of residuals vs. expected cell lengths from the GLM model for M. inermis cell lengths.
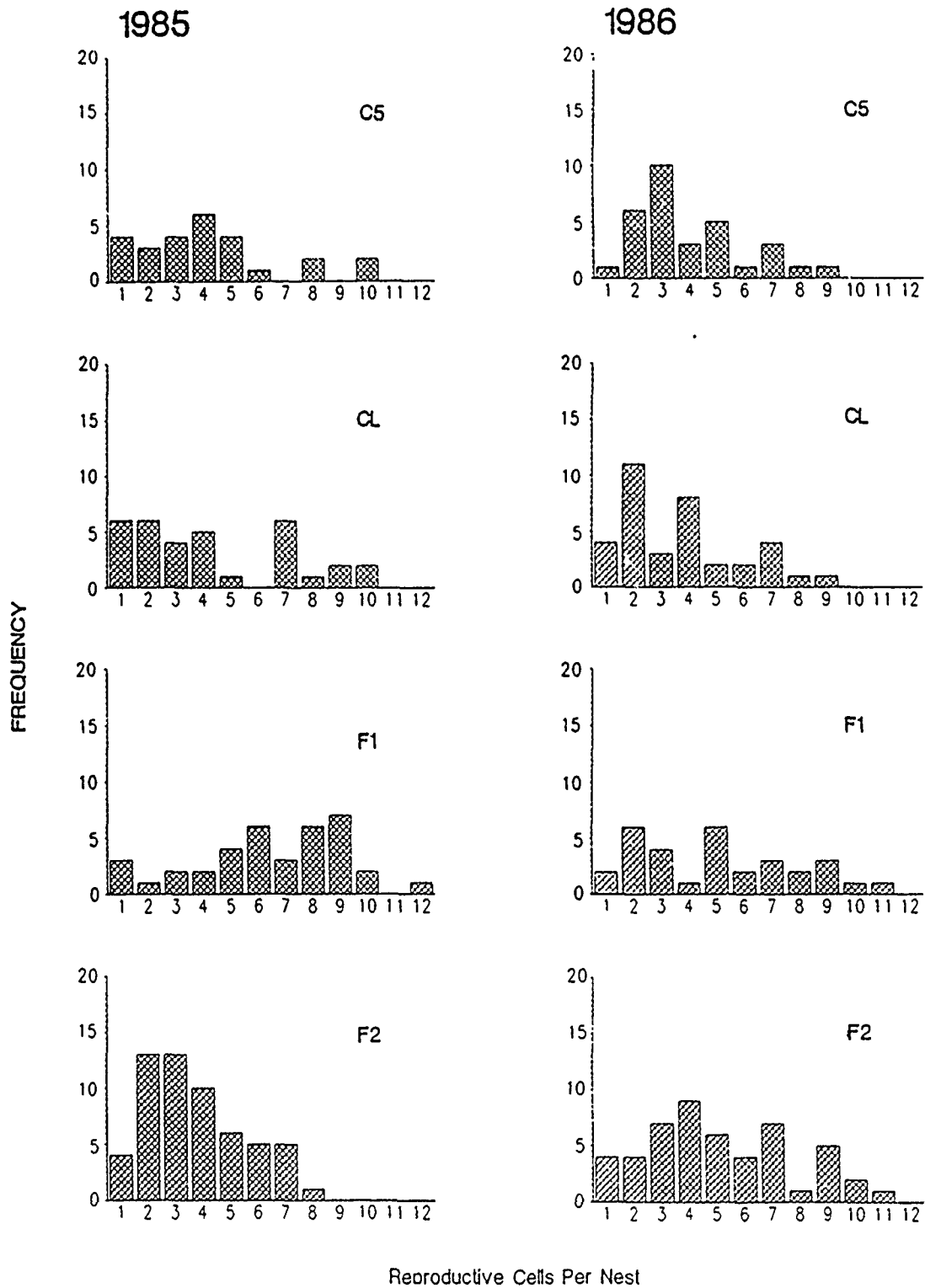
Figure 8a. Distribution of reproductive cells per nest for M. relativa, 1985-1986.
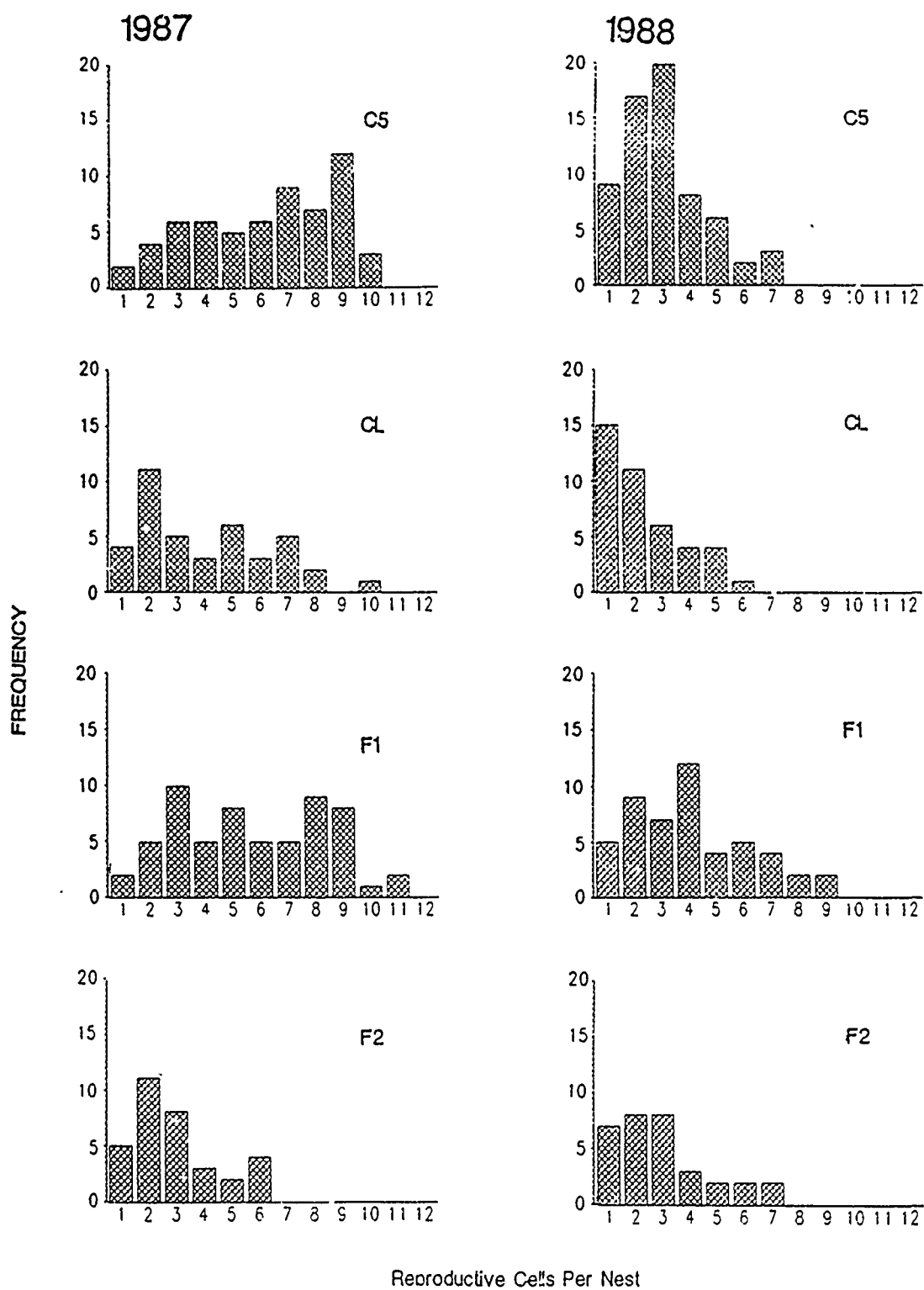
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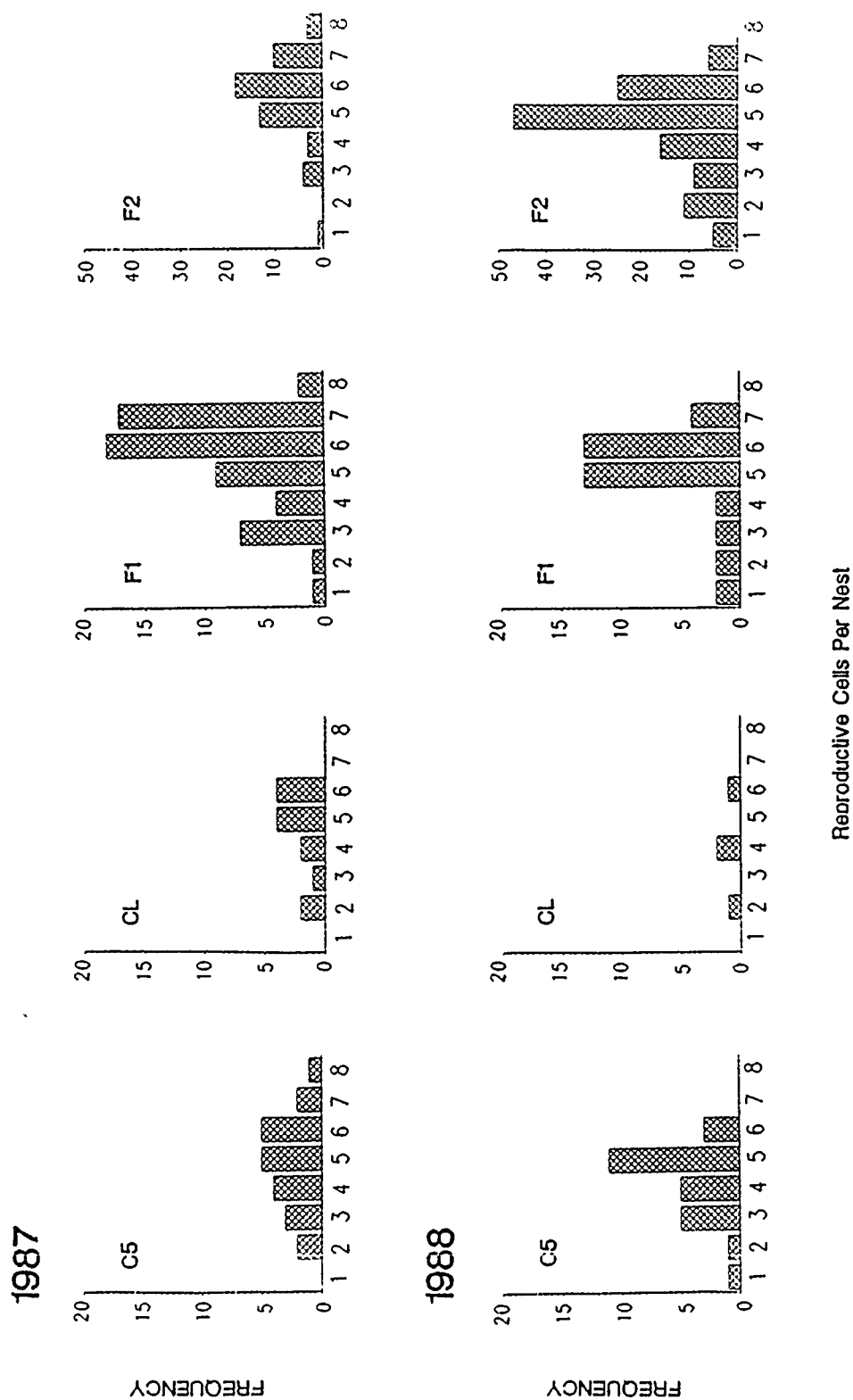
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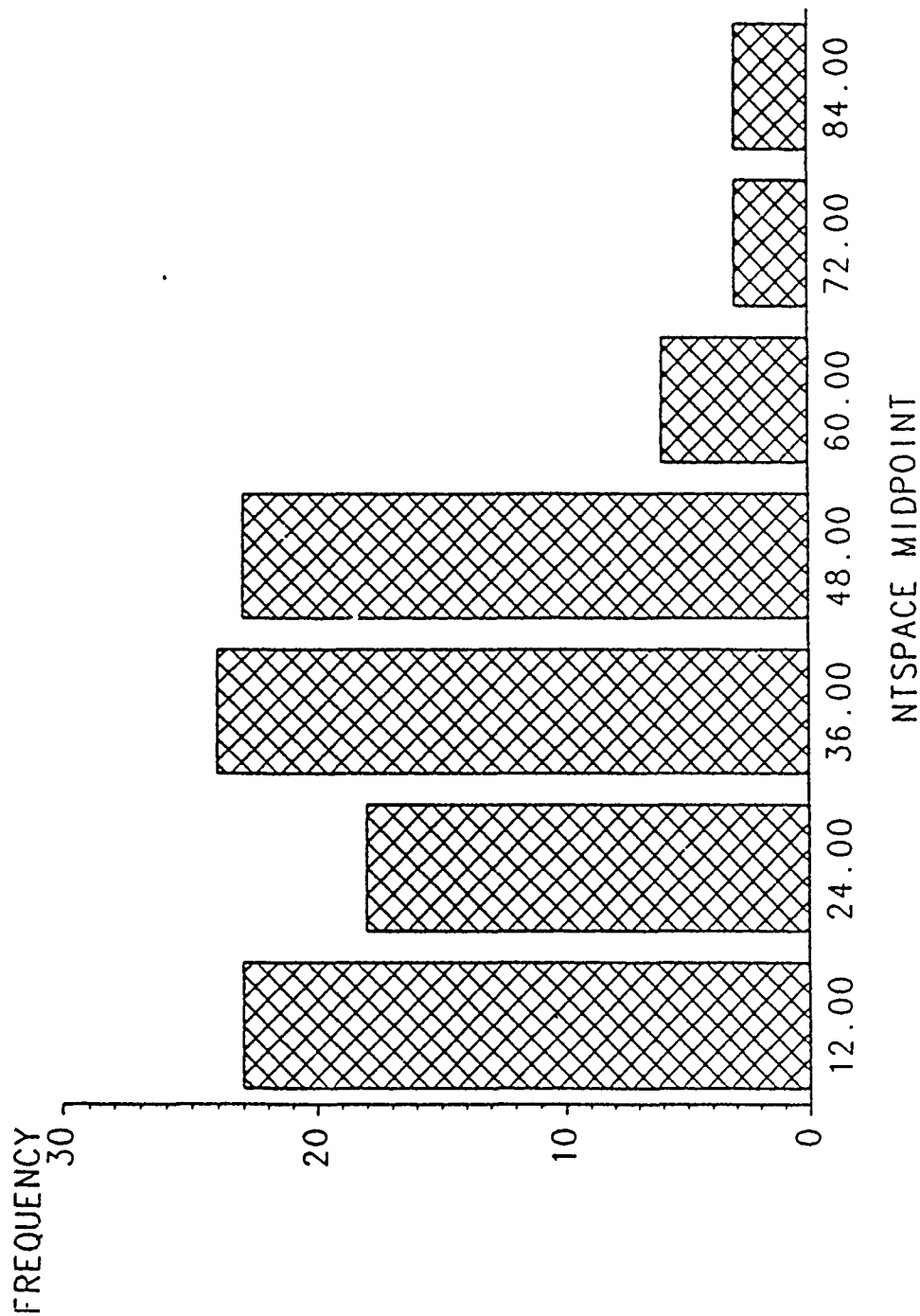
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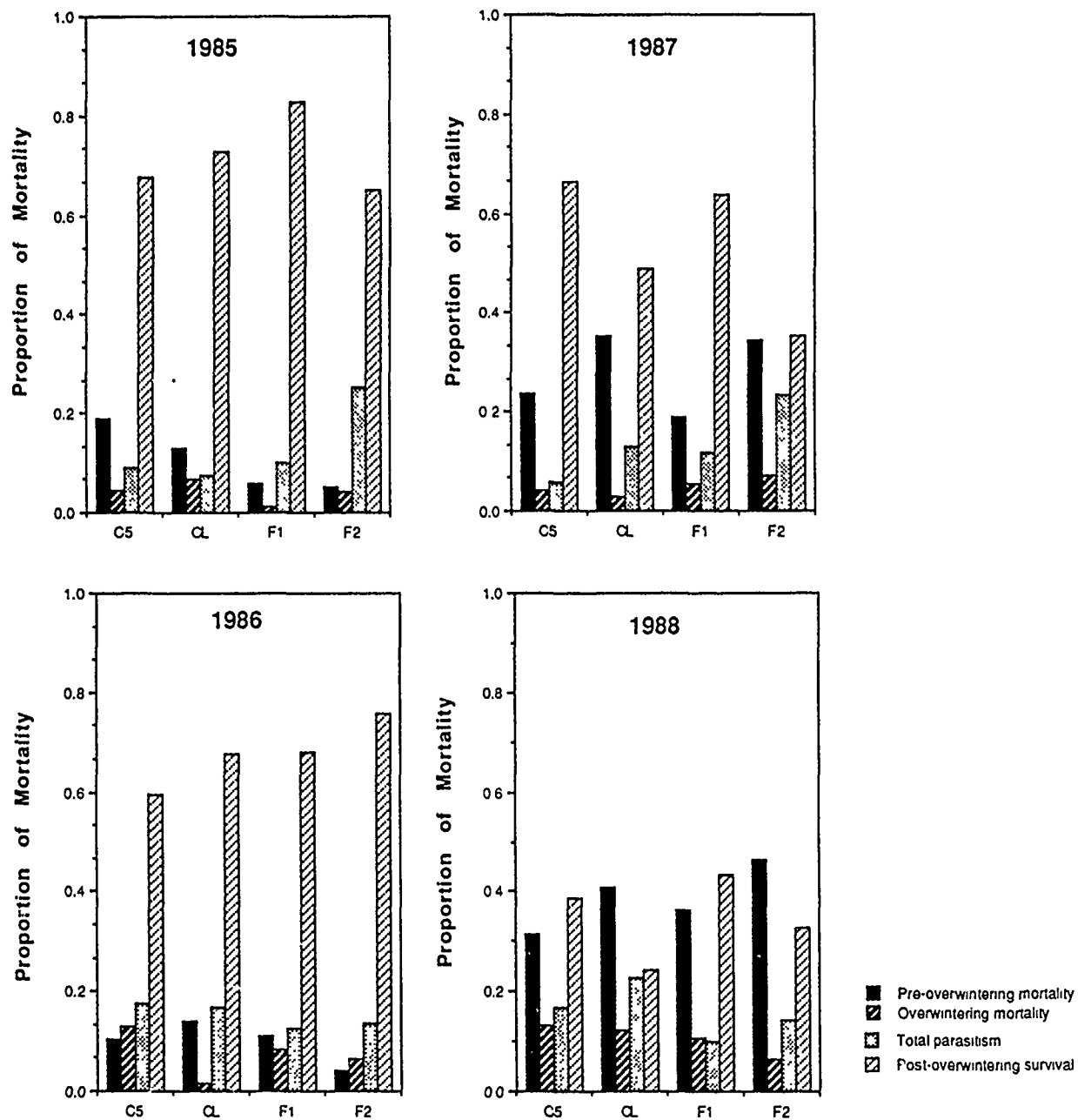
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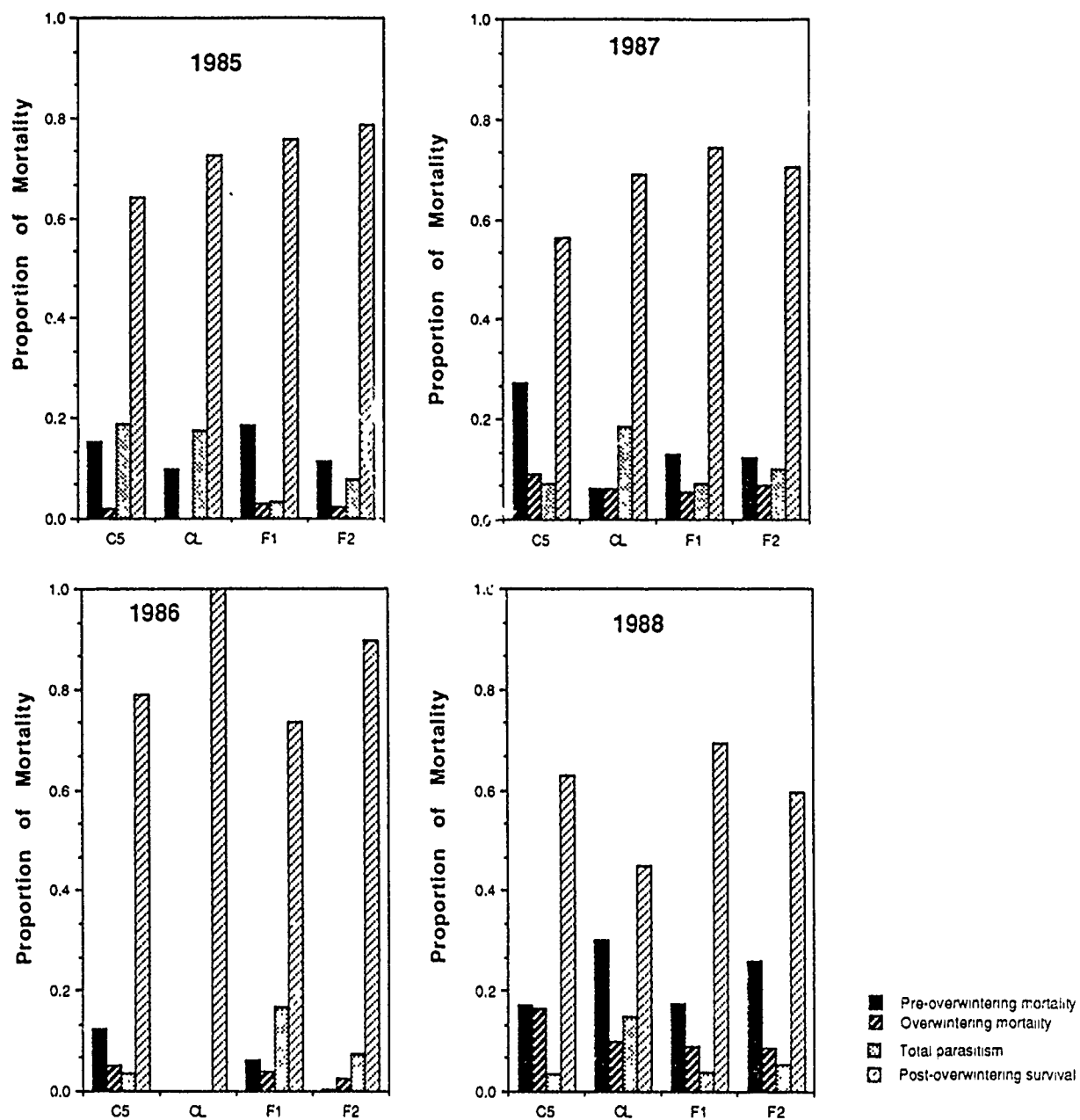
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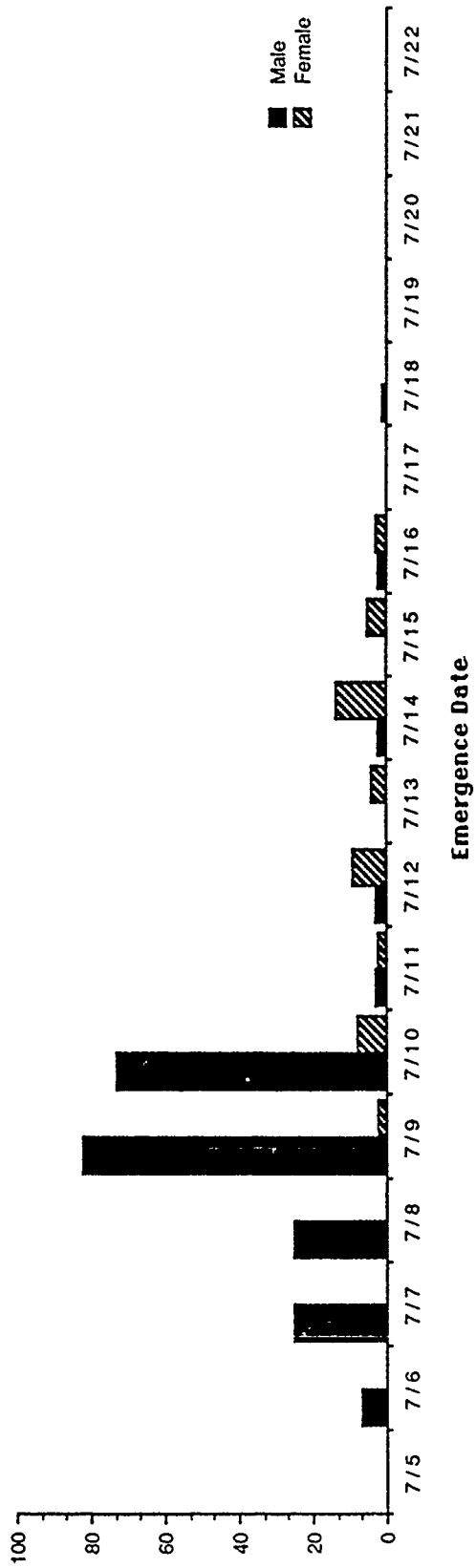
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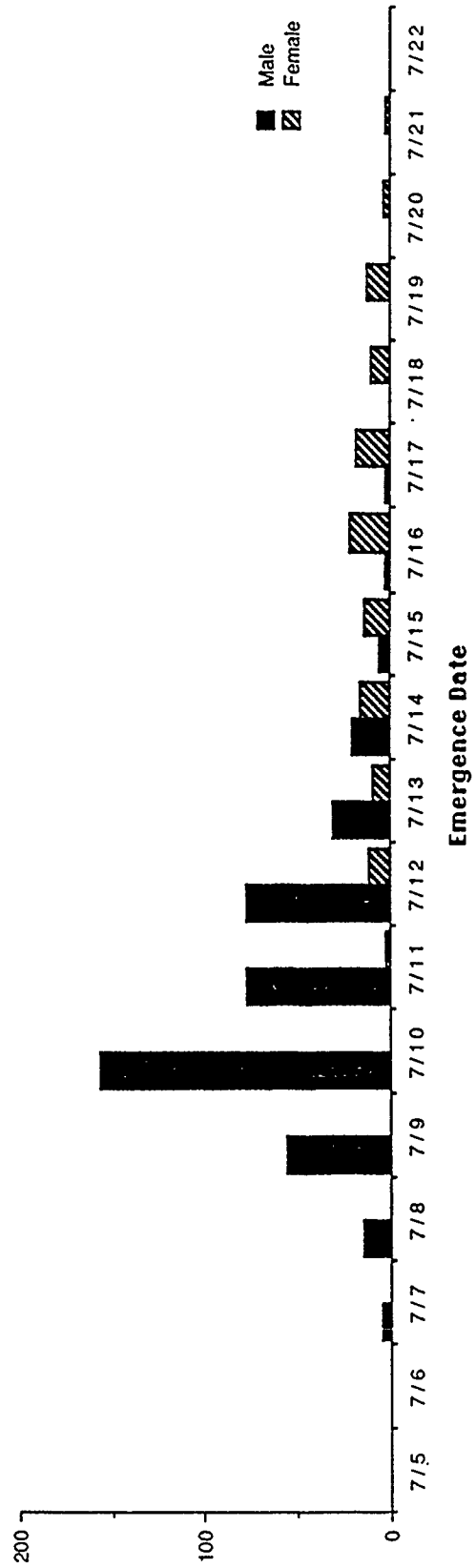
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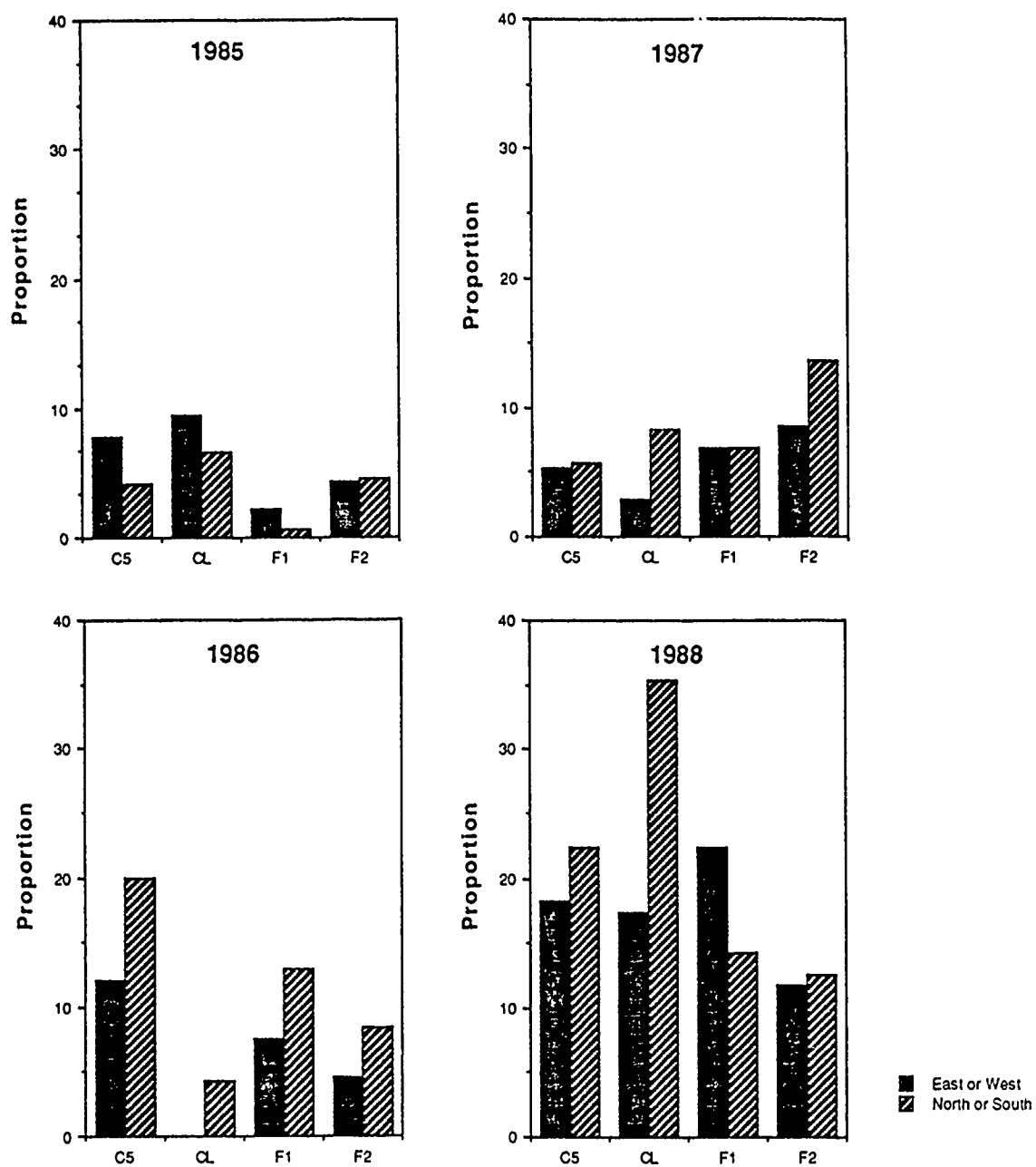
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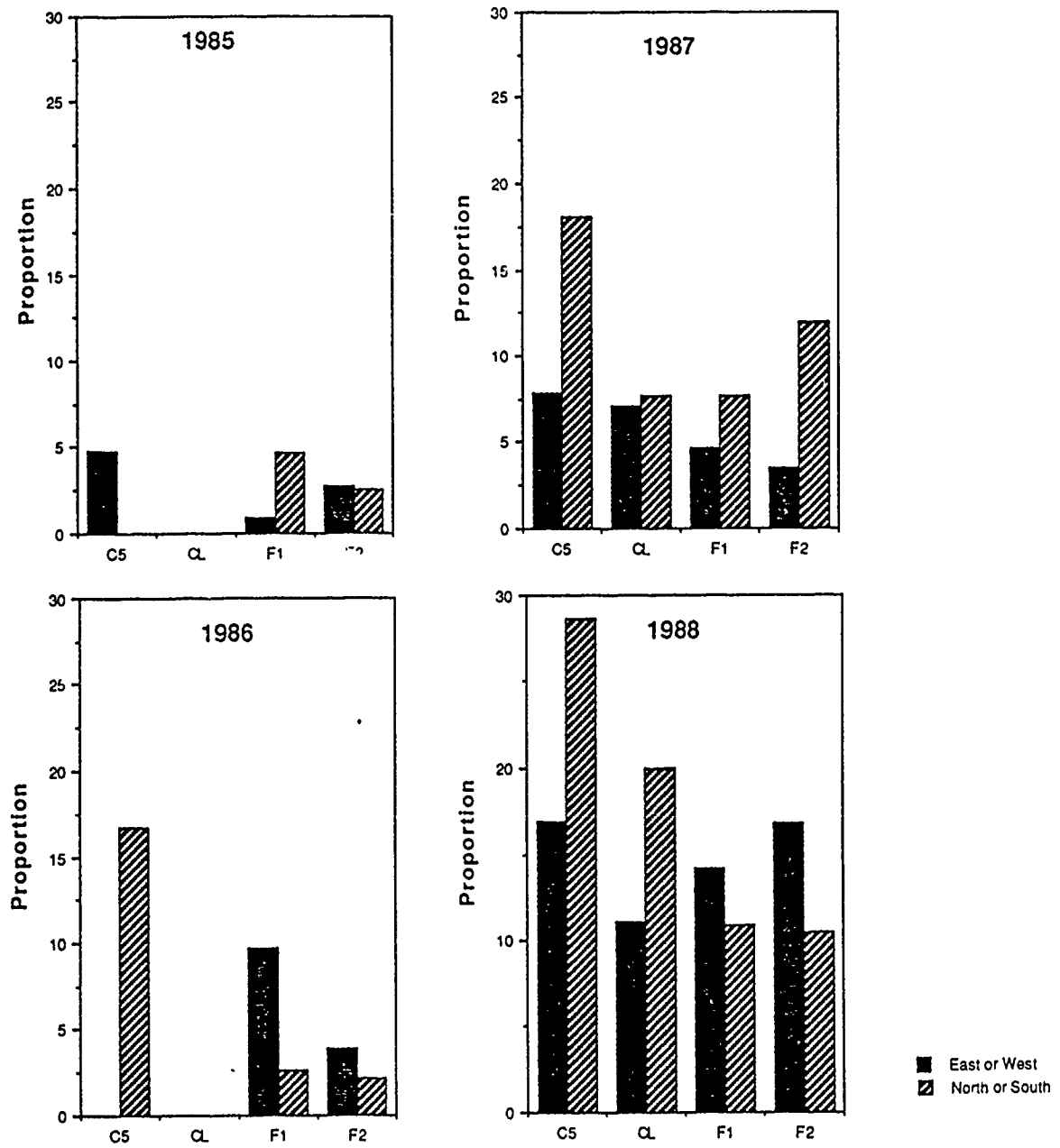
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ELF COMMUNICATION SYSTEM ECOLOGICAL MONITORING PROGRAM

SMALL VERTEBRATES: THE MICHIGAN STUDY SITE
TASKS 5.6, SMALL MAMMALS, AND 5.12A, NESTING BIRDS

ANNUAL REPORT: 1989

Subcontract No.: EC6595-88-C-006

Subcontracted to:

THE BOARD OF TRUSTEES, MICHIGAN STATE UNIVERSITY

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Releasing Authority: _____ Date: 5/23/90
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                          Contract and Grant Administration
                or   RICHARD L. HOWE
                      Assistant Director
              Contract and Grant Administration

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ABSTRACT

The small mammal and nesting bird biological studies in the western Upper Peninsula of Michigan for the year 1989 are reported. Previous years' data include base-line data from 1983-1985 and data collected during partial antenna testing from 1986, 1987 and 1988.

Small mammal community studies were dropped due to budget constraints.

Data on tree swallow fecundity, survival and growth were analyzed across all years. There were no differences for most measures between plots near the antenna (test plots) or plots farther away (control plots). Variables found to be similar among plots were clutch size, distribution of clutch size, likelihood to hatch, hatch rate, likelihood to fledge, number fledging, age at eye opening and feather eruption. These variables show year effects due to weather but no effect due to the antenna operation. Mortality of eggs and nestlings was the same on test and control plots. Overall nest mortality, incubation phase and nestling phase mortality were also the same for test and control plots. Growth rates of tree swallow nestlings for body mass, leg length (tarsus), arm length (ulna) and wing length showed no difference between test and control plots, except for tarsus which this year grew more slower on the test than on control plot, for unknown reasons. The age at half of growth showed no difference between test and control plots for these same variables. All growth variables were very different among nests.

Growth rates of young deermice were similar between test and control plots as were ages of eye opening and incisor eruption. A significant effect of the nestlings mother has occurred every year.

Tree swallow homing studies continued to show greater numbers of displaced birds returned to test than control plots. The time required to return to the

plot was also less for te.. than control birds and overall times were shorter than in previous years.

Small mammal homing studies indicated no difference in frequency of return for chipmunks, but more deermice returned on test than control plots.

Developmental abnormalities were not different in number on test and control plots in 1989. A new measure, egg volume, was also not different among plots.

Maximum aerobic metabolism was similar on plots for deermice and chickadees. For chickadees, this is the first year we have not seen a plot effect. Earlier years, chickadees from test plots had lower maximal metabolic rates.

SUMMARY

The 1989 report contains results from the biological studies of small mammals and birds from the time period preceding antenna testing (1983 to 1985) and the partial antenna testing years of 1986, 1987, 1988, and the full operational strength of 1989. While findings must be considered as incomplete until the end of the project in 1992, each year's data is useful in establishing trends in the aspects of small mammal and nesting bird biology at the study sites.

In all years, nesting tree swallows on both test and control plots laid clutches of similar size with a similar likelihood to hatch and fledge on test and control plots. Mortality of eggs, nestlings and nests taken over both stages of nest life were higher on test plots in years past, but not in 1989. Growth and maturation (eye opening, feather appearance) of nestling tree swallows showed no difference on test and control plots, with the possible exception of leg growth. This trend continues findings of earlier years. As in previous years, parental care seems to greatly influence nestling growth and parents differ greatly in their ability to raise their young. These differences are obvious on test and control plots. Growth and maturation of deermice showed no difference between test and control plots. As with the tree swallows, mothers showed large differences in their ability to raise their offspring.

Homing studies of tree swallows continued to show higher rates of return and faster return times for birds from test plots. Overall, times to return were shorter on both test and control plots compared to previous years.

Chipmunks homed had similar return rates on test and control plots, however, deermice showed a greater likelihood to return to test plots than controls, a new finding for 1989.

Abnormalities of tree swallow embryos showed no difference in frequency between test and control plots. A new measure was used this year to compare eggs, a volume measure. No difference between test and control plots was found for this measure.

Maximal metabolism of deermice and chickadees showed no difference for test and control plots. This is the first year that chickadees have not shown a plot effect (lower on test plots).

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PREFACE

This report begins with an extensive statement of the rationale for the studies proposed (see next section, titled "Rationale for Proposed Studies"). Then a section is provided on the overall research design and research facilities. Individual elements of the work are then described in detail in a series of subsequent sections. Each of the sections on individual work elements consists of three parts: (1) a brief restatement of the purpose (rationale) for the work, (2) a detailed description of research methods, and (3) a presentation of representative results gathered during prior years. The presentations of results include discussions of statistical sufficiency, including projections of the sample sizes required to discriminate between test and control plots in future years.

RATIONALE OF STUDIES

Dozens of species of small birds and mammals are resident near the ELF

Communication System, in the upper peninsula of Michigan, and the operation of

the Communication System could in principle affect any of them in any of

countless ways. Even with virtually unlimited resources, it would be

impossible to monitor individually all ecologically important aspects of all

species for possible effects of the Communication System. Accordingly, we

have had to exercise informed judgment in selecting variables for study. In

this process, we have been guided by one overriding goal.

Our major goal has been to focus much of our effort on attributes of

individual animals that are particularly likely to be susceptible to

perturbation by the ELF Communication System. The reason for this focus is

that laboratory research indicates that if the ELF Communication System is to

have effects on birds or mammals, the effects will likely be small, and thus a

statistically robust experimental design will be required to detect them (AIBS

1985). Large numbers of independent measures can be readily obtained on

individual attributes, thus facilitating statistical detection of even small

effects that the ELF Communication System might have.

In our studies of attributes of individual birds and mammals, we emphasize

ecologically significant variables that are especially likely to be

susceptible to perturbation. Reproduction and development, for example,

receive particular attention because they not only are demographically

important but also are more likely to be sensitive to adverse environmental

changes than many other animal properties (e.g., Goodposture 1955, Koskimes

1950, Kluijver 1951, Krebs 1971, Lack 1954, 1966, Nice 1954, Perrins 1965,

Perry and Rowlands 1973). Behavior is studied in depth because it is

sometimes modified readily and such modifications can have major repercussions on the lives of individuals and populations (e.g., Cohen et al. 1980, Green 1979, Morse 1980, O'Connor 1978, Slobodkin 1968).

In the following paragraphs we describe in detail the rationale for each aspect of our work on individual attributes. This work is concentrated on four particularly abundant species. The species have been carefully selected with a view to maximizing their ecological and taxonomic diversity, so as to maximize the probability of detecting whatever diverse effects the ELF Communication System may have. The four are the tree swallow (Tachycineta bicolor), the woodland deermouse (Peromyscus maniculatus gracilis), the black-capped chickadee (Parus atricapillus) and the eastern chipmunk (Tamias striatus). To facilitate readability in the remainder of the report, they will be referred to simply as the "tree swallow", "deermouse", "chickadee" and "chipmunk", respectively.

Behavioral Studies

In view of the established sensitivity of certain types of orientational behavior to alteration by the ELF fields (e.g., Graue 1974, Keeton et al. 1974, Larkin and Sutherland 1977, Southern 1969, 1971, 1972a, 1972b, 1973, 1974, 1975, 1976), orientation and homing in the tree swallow, deermouse, chipmunk, and certain other mammals are being tested to see if they are affected by the ELF Communication System. Specifically, the ability of animals to return to their home-range or territory after displacement is being assessed. We know that animals are able to find food (Krebs 1971, Royama 1966) and escape predators (Metzgar 1967, Watson 1964) more effectively in their home-range or territory than in less familiar areas. Thus, any

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disturbance of their ability to return to their home-range or territory after wandering afar could decrease their probability of survival.

The attentive behavior of parental tree swallows and deermice is being assessed by monitoring visits to the nest containing eggs and young. Disturbance of attentive behavior by the ELF Communication System, if it occurred, could impair development of eggs or nestlings inasmuch as the latter are dependent on parents for both food and warmth (e.g., Balen and Cove 1972, Hill 1972b).

Reproduction, Growth, and Development

The frequency and type of prenatal developmental abnormalities are examined in tree swallows. Mammals are not studied in this respect because reproductive females would have to be killed to examine fetuses, and such deaths could have serious, adverse effects on population demographics. Prenatal developmental stages are especially likely to be susceptible to perturbation (Axelsson 1954). Developing avian embryos have two major periods of sensitivity (Hamilton 1952) which occur during the first 4 days following the onset of incubation and the period just prior to hatching. A majority of the spontaneously occurring developmental abnormalities manifest themselves during these two periods (Riddle 1930, Hutt and Pilkey 1930, Hutt and Greenwood 1929, Hutt and Crew 1929, Landauer 1943, Martin and Insko 1935, Hamilton 1952). During these periods, the embryos are sensitive to changes in naturally occurring environmental agents such as temperature, humidity, CO_2, and O_2 (Alsop 1918, Babott 1937, Pembrey et al. 1894, Romanoff et al. 1938, Taylor et al. 1933). Additional teratological agents include vitamins and their antagonists (Cravens 1952), hormones (Zwilling 1956), alcohol and ether (Stockard 1914), metal ions (Ridgeway and Karnofsky 1952), narcotics (Reese

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1912), various forms of radiation (Windle 1893, 1895, Gilman and Baetjer 1904, Hinrichs 1927, and Dixon 1952) and physical jarring (Stiles and Watterson 1937). There is, at present, no evidence to demonstrate that electric and magnetic fields of the magnitude generated by the ELF Communication System are capable of directly causing embryonic or fetal developmental defects. However, indirect effects are possible. Should the incubation behavior of parent birds be disturbed by the ELF Communication System, developing eggs might suffer developmental abnormalities by virtue of experiencing abnormal reductions or fluctuations in temperature. (Zwilling 1956, Hamilton 1965).

We monitor aspects of fecundity in both tree swallows and deermice. In the birds, we count the number of eggs produced per female and the number of viable eggs and young per clutch. In the mice we monitor numbers of young per litter. Fecundity is an important variable to study not only because it is demographically significant but also because it reflects on a number of variables that could, in principle, be affected by the ELF Communication System. Alteration of male or female reproductive physiology could affect fecundity. Further, any serious disturbances of prenatal development in mammals or birds would likely be reflected in a decrease in fecundity inasmuch as abnormal embryos frequently fail to be born (i.e., they are resorbed in utero or fail to hatch) or are eaten or discarded by the parents soon after birth.

Postnatal mortality and the growth and development of nestling tree swallows and deermice are also followed. Any effects that the Communication System might exert on the young themselves could be reflected in altered rates of mortality, growth, or development. Alternatively, disturbances of parental attentive behavior could be influential because the rates of mortality, growth, and development of nestlings are dependent on the extent to which

parents provide food and warmth (Hill 1972b). The size of nestlings at the time of weaning or fledging is of particular interest because when young become independent of their parents, they must become substantially self-sufficient and their maturity can affect their likelihood of survival. Evidence exists that young birds that are of relatively small size at fledging are significantly less likely to survive than ones that grow to larger size while in the nest (Lack 1966, Murphy 1978, Perrins 1965).

Maximal Aerobic Metabolism

In the region of the ELF Communication System, low temperatures make winter the most physiologically stressful time of year, at least for animals such as chickadees that live wholly or predominantly above the snow. We study physiological variables that affect the ability of chickadees and small mammals to cope with the severity of the winter climate. Deficits in the physiological ability to cope would be expected to decrease the probability of survival to the next reproductive season.

Birds and mammals keep warm in cold environments by producing heat metabolically to offset heat losses. The extent to which they can keep their body temperature above air temperature depends on how rapidly they can produce heat. In other words, the lowest air temperature at which they can maintain their usual body temperature is a function of their maximal rate of aerobic metabolism (= heat production) (Hart 1957). In view of these principles, we measure the maximal rate of aerobic metabolism of chickadees and deermice during winter. This peak rate of heat production is informative not only because it determines the lowest air temperature at which thermoregulation is possible but also because it likely provides an index of metabolic endurance. The higher an animal's maximal rate of heat production is, the longer the

animal will be able to maintain any particular submaximal rate of heat production (Astrand and Rodahl 1977, Wickler 1980). Endurance is important because low air temperatures demanding high heat production can persist for long periods of time.

Beyond its immediate significance for survival in a cold climate, the maximal rate of aerobic metabolism is a valuable variable to measure because it provides an index of physiological health. In fact, peak aerobic metabolism is wide. used as such an index in studies of humans. In their classic Textbook of Work Physiology, Astrand and Rodahl (1977) state that "the maximal oxygen uptake is probably the best laboratory measure of a person's physical fitness" if by fitness we mean the capacity of the individual for prolonged heavy work. Brooks and Fahey (1984), in the best of the recent texts on human exercise physiology, state that the maximal aerobic metabolism is "a good measure of fitness for life in contemporary society". Just as peak aerobic metabolism is used as an index of fitness for humans, it can also be so used in studies of animals. A deficit in the peak metabolism among individuals living near the ELF antenna would indicate that some attribute of the all-important systems involved in oxygen supply and use has been adversely affected by the ELF electromagnetic fields. Additional tests would then be required to determine the particular attribute(s) affected. The ability of the respiratory system to provide oxygen, the ability of the circulatory system to transport oxygen and nutrients to metabolically active tissues, the ability of storage tissues (e.g., adipose tissue) to mobilize stored nutrients, and the enzymatic competence of metabolically active tissues to catabolize nutrients are among the variables that influence an animal's peak rate of aerobic metabolism (Wang 1978). In human studies, peak aerobic metabolism is usually elicited by having individuals run on a treadmill. We

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elicit peaks by exposing animals to cold, in part because the method is

technically simpler than treadmill running (given that animals require

extensive training to use a treadmill successfully) and in part because the

cold-induced peak is of immediate relevance to understanding winter ecology.

OVERALL RESEARCH DESIGN AND SUPPORT FACILITIES

To detect possible effects of the ELF Communication System, we compare animal attributes on test plots (test sites) with those on paired, spatially separated control plots (control sites).

Test plots, as specified in the original IITRI Request for Proposals, are areas close enough to the Communication System that electric and magnetic fields attributable to the System, and measured in the soil near the earth's surface, will approximate 0.07 volt/meter and 0.03 Gauss, respectively. Furthermore, electric and magnetic fields attributable to ELF sources other than the System are to be at least an order of magnitude lower than those attributable to the System.

Control plots, according to the original Request for Proposals, are areas sufficiently distant from the Communication System that electric and magnetic fields attributable to the System, measured in the soil near the earth's surface, are at least an order of magnitude, and preferably two orders of magnitude, below those at paired test plots. Furthermore, electric and magnetic fields in the air and earth attributable to ELF sources other than the System (especially 60 Hz sources) are not to differ by more than an order of magnitude between the control plots and their paired test plots.

For purposes of experimental design, the test plot(s) used for any particular work element are paired with particular control plot(s). The plots of a pair are matched as closely as possible for vegetation, soil type, drainage, and other such features. By pairing plots in this way, we minimize the likelihood that non-ELF differences between plots will introduce significant confounding effects into our results.

A major strength of our research is the paired plot design. Within a year, we can compare possible ELF effects across plots. The design has an

additional strength due to the capability of before and after comparisons for each plot where each plot can be used as its own control through time. We consider three phases of antenna operations: 1) pre-antenna, 1983-1985, 2) antenna testing, 1986-1988, and 3) full antenna operation, 1989-1991.

Different work elements are carried out on different pairs of plots for several reasons. Specific work elements could interfere with other work if both were carried out on the same populations of animals; areas where we artificially remove animals (e.g., bird embryos), for example, are not used for research on natural populations. Another factor that demands the use of different plot pairs for different work elements is that the various species we study do not all occur in similar habitat types; field habitats are required for the swallows, whereas forests are required for the deermice.

To minimize potentially confounding differences between test and control plots, sham corridors have been cut through the forests at the control plots. These corridors are clearings of the same width as the corridors cut for installation of the Communication System antenna near test plots. They were cut with similar equipment, and they have been treated similarly after cutting. In brief, the sham corridors are as identical as possible to the antenna corridor except that antenna poles and wires have not been installed in the shams. Areas for animal study on control plots and those for animal study on test plots are located about the same distance from the sham corridors and antenna corridor, respectively.

Plots were established as matched pairs (Table 1) of test and control plots for the various work elements. Test plots were located along the north-south antenna element and control plots were located at varying distances to the west of the antenna (Figure 1). The names given to the plots (Table 1) are

the standardized ones we use in all our descriptions of experiments and results. The alpha-numeric codes for plots are those used by IITRI.

Modifications in Project Scope and Statistical Sufficiency

The number of study elements was reduced in March, 1989, when budget cuts were made to meet increased wages of non-faculty employees on the project. The wage increases resulted from a labor settlement at Michigan Sate University. The following research elements were <u>dropped</u>: small mammal community studies, small mammal parental care, and tree swallow incubation. All remaining research elements were continued at full strength.

We have revised our standards for statistical sufficiency for the research program based on our years of experience with the various study elements to date. We originally established the standard of statistical sufficiency in our work that we predicted would provide a 90% certainty of detecting a 20% difference between test and control sites at the 5% level of significance. While we can still meet these standards on some of our work, we clearly can not for others, such as growth of both tree swallows and deermice (see Tables 35, 36, 39). Variation among nests unrelated to plot is the principle reason. These unexpectedly high variances lead to projections of sample sizes beyond the possible scope of our research. We must therefore relax our standards of statistical sufficiency. We have decided to report the actual level of detectable difference in means achieved in a test and the difference we could detect if we relaxed the level of certainty (power) to 70%. The reader will therefore be able to judge for each test the particular statistical confidence that can be met. Literature values for detectable differences or power are not currently known to us for comparison. It seems that most authors do not report either value. For discontinuous variables, we have used different

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procedures to determine sample size (see Gill (1978, p. 82)). Therefore we do not propose changes in statistical sufficiency since we appear to be able to meet the stricter requirements with these data. Discussion of sample size and power of test are presented with the data for each study element (see below).

Our base of operations for the on-site field and laboratory studies is a large house rented in Crystal Falls, MI (801 Crystal Ave.). The physiology laboratory is installed there. The holding facility for temporary housing of animals used in the physiology experiments is located approximately 3.5 miles south of Crystal Falls, MI in an area with minimal electromagnetic interference. We have a shop for construction and maintenance of field equipment and a large shed for storage of traps, cages, construction materials, and seasonal field equipment. We also have a well established data management system housed there (see below), and living space is provided for employees. We rent and maintain three pick-up trucks to provide transportation between our base of operations and field research sites in all weather conditions on a year-round basis. In addition, we rent a snowmobile to gain access to our more remote sites during winter and spring when traveling the entire distance by truck becomes impossible.

For data management we employ an IMS (now LF Technologies) computer system at the MSU Museum in East Lansing. The system is multi-user and allows storage of data on fixed and removable media. Zenith (AT-class) computers are used at the field laboratory in Crystal Falls. Data transfer and analysis are accomplished using both systems. Field data are collected by NEC PC-8201A portable computers. We have developed software to standardize and error check field data as it is recorded. Collected data are transferred directly into an AT computer at the field laboratory each day. Transferred data are

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immediately edited and stored on removable and fixed disks for later analysis. Certain data are analyzed as soon as they are collected. This data management design allows us to collect and analyze large amounts of data very efficiently and accurately. In addition, in 1987, we added high speed tape backup systems to aid in recovery of data should either computer system fail, and for archiving the now voluminous data sets for the various study elements. The large sample sizes required in many of our study elements necessitate the careful and accurate data handling the system provides.

Other major equipment is described in connection with individual work elements in the sections that follow.

Measurements on 60 and 76 Hz fields

· Engineers provided by IITRI have measured 60 Hz electric and magnetic field intensities every year starting in 1983 on our test and control plots, and all the pairs we now use adequately meet the standards for field intensities already described. Electric and magnetic fields produced by the antenna system (76 Hz) were measured starting in 1986, when low amperage testing began. Measurements have continued as the antenna has become operational. A summary of the data 1983-1988 is provided in Tables 2 - 9. Data are not yet available for 1989. Details of the results of the field-intensity measurements and the measurement techniques can be found in Enk and Gauger (1985), Brosh et al. (1985 and 1986), and Haradem et al. (1987, 1988 and 1989). Earlier discussion of measures and plot pairings are outlined in the 1984 annual report (Beaver et al. 1985, pp. 3-9).

In all years, measures were made in September or October by IITRI personnel on our test and control plots during antenna operation. The distribution of operation hours by month for 1986, 1987 and 1988 for the

north-south and east-west antennas were concentrated in the months of June

through November in 1986 and 1987. Year around operation began in 1988

(Figure 2). During these years, the amperage of antenna operation varied from

3 to 75 amperes. Schedules of research activities fell within the times of

heaviest antenna operation in all years (Figure 2). Operation of the MTF was

conducted on a 33% time rotation schedule in which the east-west antenna was

on for 5 min, then the north-south antenna for 5 min, followed by both

antennas off for 5 min. The percentage of time the MTF was on varied from

1.8% (1986) to 11.8% (1988) (Figure 3).

60 Hz Fields

Table 1 provides reference to site codes used in the tables which follow.

Measurement of background 60 Hz fields (generated by existing power lines) on

control and test plots began in 1983. Transverse electric fields were

initially at or near the lower limits of measurability (Table 2). All values

were <0.001 v/m or equaled 0.001 v/m for all sites and plots, except for test

plots in 1987 and 1988. Values for transverse electric fields were about 34

times higher on test compared to control plots in 1988, continuing a trend

first seen in 1987. Apparently the presence of the antenna couples to and re-

radiates 60 Hz fields from power lines (Gauger, personal communication).

Longitudinal electric and magnetic 60 Hz fields (Tables 3 and 4) were

consistently higher on test compared to control plots. Ratios of the means

for test and control plots for each field varied from 1.0 to 32. The

difference in the strength of these fields varied with the year. Longitudinal

electric fields averaged highest on controls in 1984 and on tests in 1988.

Magnetic fields remained relatively constant on controls but increased during

1986, 1987, and 1988 in conjunction with construction and testing of the

antenna system.

Among sites within the control plot, 1C1 and 1C3 (Michigamme North and South) were consistently higher for 60 Hz longitudinal electric fields (Table 3). Sites within the test plot labelled 1T5 and 1T6 (Ford River North and South) were higher than other test sites in most years (Table 3). Magnetic fields show larger values for site 1C6 but no patterns in other control sites. Sites 1T2-1T6 all increase a large amount in 1986, 1987, and 1988 (Table 4). Site 1T1 shows a smaller increase in these years.

The control release location (1D3) and Panola Plains control site for tree swallow homing shows small differences in field strength for electric and magnetic fields (Table 5). However, much larger ratios appear on test release locations (1D1 and 1D2) and their corresponding test sites for transverse and longitudinal electric fields, with the exception of 1D2 and 1T4 in 1986 (Table 9).

76 Hz Fields

In 1986, 1987 and 1988, measurements were made on 76 Hz fields produced by the antenna during testing. Variation of 76 Hz fields was examined within a plot (among sites) to see if they were homogeneous. Control sites were all uniform with respect to transverse electric (Table 6) and magnetic fields (Table 8). For longitudinal electric fields (Table 7), sites 1C1 and 1C3 were significantly greater than 1C4 and 1C6.

Among test sites, 1T5 was greater than other sites for transverse electric fields (Table 6), and 1T6 was greater than other sites for longitudinal electric fields (Table 7). No other patterns emerged. The control sites 1C1 and 1C3 are closer to the antenna system by several Km, perhaps explaining their higher values. Test site longitudinal electric fields differ from each other because of varying distances to the antenna wire and because of variations in soil conductivity between and across sites.

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Longitudinal electric and magnetic 76 Hz fields were significantly different for test and controls (Tables 6-8), indicating that low amperage testing produced a "treatment" condition on test plots, compared to controls. For this reason, we must consider 1985 as our last pre-operation year.

The release sites for tree swallow homing studies compared to their respective study plots show low ratios for control sites and higher ratios for test (Table 9). Ratios generally increase from 1986 to 1987 but not in 1988 even with 75 amperage testing. This pattern will be examined again in relation to tree swallow homing results for 1989.

Comments on Ambient Monitoring

We have elected to use weather station data from several nearby sites to monitor the effects of climatic conditions impinging on the plots. The plots are relatively close to each other and therefore experience the same major weather patterns. Minor differences probably exist due to variations in storm tracks, local topography and vegetative features. These differences will produce some degree of variability in response in our study animals, but in most cases we expect this to be small and random in direction. It is therefore our judgment that the greatest value of station weather data will be for examination of year to year effects, rather than within a year among plots.

There is one instance where we have become aware of an effect that is probably based on micro-climatic differences among the plots. The abundance of aerial insects that are preyed upon by tree swallows appears to be greater on test plots, and less affected by cold weather, than on control plots. To examine and hopefully correct for this effect, we have instituted a program to sample aerial prey, in cooperation with Dr. D. Hussell in Ontario, Canada.

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The program is given in greater detail below in the sections dealing with
population statistics and growth of tree swallows.

STUDY OF SMALL MAMMAL COMMUNITIES

I. Purpose

The small mammal community study was not conducted in 1989. As we have stated in previous annual reports, differences between plots from year to year appear to be site specific and variable. Such variability does not allow us to examine ELF effects within the levels of our stated statistical goals. Therefore we felt the year to year variability, coupled with new budgetary constraints in 1989, would not allow us to adequately detect effects due to ELF. We therefore elected not to continue the small mammal community study.

PARENTAL AND NESTLING BEHAVIOR, AND FECUNDITY, GROWTH AND MATURATION STUDIES - TREE SWALLOWS

I. Purpose

The purpose of these studies is to characterize several aspects of the reproductive process in tree swallows at test and control sites and to test for possible effects of the ELF Communication System on these variables. Specifically, the following aspects of the reproductive process are compared between test and control sites and for each site from year to year: parental attentiveness to eggs and young, numbers of eggs per clutch, hatching success within clutches, rates of g·owth and development of hatchlings, and nestling mortality. All of these work elements are described together in this one section because they are all conducted on the same populations of birds.

II. Methods

These studies were conducted in natural or artificial clearings where we have erected arrays of nest boxes. The boxes were made of cedar lumber and mounted on posts, 1.5 m above the ground. Tree swallows readily elected to nest in the boxes and tolerated considerable disturbance by investigators. ·The boxes could be opened to permit inspection and weighing of young. Sheets of high-density polyethylene wrapped around the posts prevented access by terrestrial predators.

When possible, adults were captured on the nest after incubation was completed and banded with U. S. Fish and Wildlife Service bands for identification. Since it has been shown that certain reproductive variables are affected by the age of the female (DeSteven 1978), most of our effort was placed on capturing females. In addition, as many young as possible were banded before fledging.

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Active nests were checked daily or every other day to determine the dates
that eggs are laid, how many are laid, the dates the young hatch, and overall
hatching success. During hatching, nests were checked twice daily to
determine time of hatching with greater accuracy as well as the spread of
hatching over time. Monitoring of the nests for nestling growth and mortality
then continued until all young reached 16 days of age. Young tend to fledge
unusually early if disturbed beyond day 16. Therefore, after day 16, nest
checks to estimate fledging success were done every other day to minimize
disturbance.

Egg incubation was not monitored in 1989 due to budgetary constraints.

For studies of growth and development, nestlings were weighed every other
day with a Pesola spring scale accurate to 0.1 gm. The lengths of the tarsus,
ulna, and wing (all from the right side of the body) were measured with dial
calipers accurate to 0.1 mm. Since it was impossible for one observer to
measure all nestlings we had at least two observers collecting growth data.
However, we have noticed that different observers differ slightly in their
techniques for measuring weights and body parts. Therefore we had all
observers rotate among the plots so that every nestling was eventually
measured by all observers. Regularly rotating the observers in this way has
the effect of submerging the variance in measurement, due to observers, into
the error in each nestling's growth curve. This measurement protocol
unfortunately prevents us from being able to block observer effects in the
statistical design. However, as we show below, when we use data from each
individual bird's growth curve, even the significant effects of differences in
observer technique do not prevent us from being able to detect small
differences in patterns of growth.

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For analysis of growth data, we used the procedure for fitting growth data to models of growth proposed by Ricklefs (1967) and used previously for tree swallows by Zach and Mayoh (1982). Briefly, the data for each nestling were subjected to curve fitting using an exponential or logistic model in a linear regression routine in SAS (Statistical Analysis System). The model of best fit, as judged by having the highest value of R^2, was used in subsequent analyses to obtain the rate of growth, the intercept, and the inflection point. The model of best fit every year, including 1989, has been the logistic.

In past years we have detected significant differences in growth rates of young tree swallows between test and control plots. Recent evidence suggests that food availability on a plot can have a significant effect on both clutch sizes and growth rates of tree swallows (Hussell and Quinney 1987, Quinney et al. 1986). In order to determine what degree of variation between test and control plots in growth rates is the result of food resource availability, we have undertaken steps to quantify the flying insect abundance at each site. We have communicated with Dr. Hussell of the Ontario Ministry of Natural Resources and have designed a sampling scheme based on his earlier work (see Hussell and Quinney 1987, for detailed methodology). At each tree swallow site we collected flying insects during the daylight hours in two suspended conical nets with alcohol traps. These nets were located among the nest boxes and were constructed to face passively in the wind so as to continually sample insects which either flew or were blown into the nets. Previous studies showed an excellent relationship between the insects collected in this type of system and the insects delivered to young swallows in the nest by their parents (Quinney and Ankney 1985). Sampling began before the initiation of any egg laying and ended when all young from the plot had fledged. After

insects were sorted into size classes we computed an index of the biomass of flying insects determined from daily catches on each plot. This allowed us to compare the prey abundances between test and control plots in order to look for explanations in differences in growth rates between plots not due to age of the adults or clutch sizes. These data will further refine our abilities to detect possible subtle differences in tree swallow reproductive measures due to electromagnetic fields associated with the Communication System.

III. Results - 1989

Tree swallow plot names, numbers of boxes at each plot, and percent occupancy for 1985-89 are shown in Table 10. Small differences in number of boxes on some plots will be noted when compared to earlier annual reports due to attrition or addition of boxes. With the placement of additional boxes on plots in the early spring 1989 we now have a full complement of bird boxes at test and control sites. Of the 357 nest boxes monitored in 1989, 285 (80%) had egg-laying activity which is a slight increase over activity observed in years 1985-1988. This increase is due, in part, to the additional opening caused by completed cutting of the sham corridors around the perimeter of the control plots, the roller-chopping of encroaching aspen by the Michigan Department of Natural Resources, and by our efforts at predator-proofing of nest boxes. In early spring, all of the nest box poles were wrapped with high density polyethylene sheet to help prevent access by terrestrial predators. With increased return rates of nesting adult observed each year we have established plots which will provide adequate sample sizes for all of the tasks reported on below. Starting in 1986, we conducted all aspects of the research program on specific plots established for each individual task (see Table 1) and will continue with this protocol as originally proposed.

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The age of adults breeding on the plots was quantified in earlier years by categorizing a bird as an adult if it had a high percentage of its back plumage colored iridescent green. Younger birds have mostly a gray back plumage with little green (DeSteven 1978). In 1985, we found many more young birds nesting on control than test plots (Beaver et al 1986). In 1986, we were not able to make as complete a determination because many birds abandoned their nests due to inclement weather prior to the time we designated to assess age of adults. However, we did keep records of birds we saw on our daily visits to the plots. Less than 10% of nesting birds were young birds and there appeared to be equal numbers of them on test and control. In 1987, less than 20% of nesting birds were young birds, with greater numbers of young birds on the control plots. Of the nesting birds observed in 1989, 11% were known to be young females. The younger birds were observed more frequently on the control plots (15%) than on test plots (5%). This greater number of young birds on the control plots may be reflective of an inherent difference in habitat quality between the two plots. Even if this is true, the collection of data from test and control both before and after antenna activation should enable us to sort out antenna effects and habitat effects.

Fecundity. Summarized fecundity data for tree swallows in 1989 and comparisons to 1985, 1986, 1987 and 1988 are presented in Table 11. These data were collected from the Pirlot Road test plot and Tachycineta Meadows control plot and exclude any renesting attempts. Mean clutch size in 1989 at Pirlot Road (5.1 eggs/nest) was similar to Tachycineta Meadows (5.4 eggs/nest), (t=1.17, p=0.26). Both of these values are within the range of those reported elsewhere for tree swallows (Chapman 1955, DeSteven 1978, Zach and Mayoh 1982, Hussell 1983b). When data on clutch size from the last five years are considered together (Table 13), we observe no significant effect due

to plot (F=1.72, P=0.191), year (F=1.16, P=0.33), or plot/year interaction (F=2.06, P=0.085).

We have suspected there are differences in available food at the test and the control plots and this could be influencing clutch size, a finding reported for tree swallows in Canada by Hussell and Quinney (1987). As reported in past years, we are cooperating with Hussell in determining prey biomass at our sites and we should be able to examine this using the data we have on insect biomass as soon as the analysis of our insect data by Hussell is complete. There was no difference in the distribution of clutch sizes between test and control plots during 1989 or in prior years (Table 11, G-tests of independence).

Hatching success (Table 12) was slightly less at the Pirlot Road test plot (83.3%) than at Tachycineta Meadows (83.4%) during 1989 but this difference in likelihood to hatch is non-significant (G-test of independence, Sokal and Rohlf 1981, G<0.000, df=1, P>0.90). When 1989 - 1985 data are analyzed together, likelihood to hatch is shown to be independent of both plot and year (G=12.70, df=9, P>0.10). The actual number of young that hatched per nest (Table 11) was greater on the test (4.3 young/nest) than on the control (4.2 young/nest) in 1989, these values being within the range reported elsewhere (Low 1934, Paynter 1954). When hatch rate data from the last five years are considered together in an analysis of variance (Table 14), we find no significant effects due to plot or plot/year interaction (both P>0.35). A significant difference was noted between years (P=0.012), due to higher numbers hatched per nest in 1986 and 1988. Another incident of inclement weather during 1989 was responsible for the lowest fledging rates observed thus far during five years of study. Temperatures far below normal coupled with precipitation during 9-15 June caused many complete nest failures and

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only a small proportion of nests survived with no mortality of young. Most nests had hatched young within the few days prior to this cold snap and the nestlings had not reached an age when they could begin effective thermoregulation. Due to the total lack of aerial insect prey, many adults abandoned the nest and subsequently the young died of exposure. Many eggs still being incubated at this time were also abandoned and failed to hatch.

Fledging success was less at Pirlot Road (17.6%) than at Tachycineta Meadows (22.2%), although these differences in likelihood to fledge are not significantly different (G=0.76, df=1, P>0.3). When 1985-1989 fledging data are examined together (Table 12), likelihood to fledge is shown to be highly dependent upon year and plot location (G=316.55, df=9, P<0.001). When this 10 X 2 table is broken down into its components of plots (test and control pooled over five years) and years (five years pooled over test and control plots), there are no detectable plot effects (G=1.86, df=1, P>0.1), but there are significant year effects (G=286.1, df=4, P<0.001) which are due to the greatly decreased likelihood to fledge associated with adverse weather following hatching in 1986 and 1989.

The actual number of young fledging per nest during 1989 (Table 11) was less at the Pirlot Road test plot (0.8 young/nest) than at Tachycineta Meadows control (0.9 young/nest). When data on actual numbers of young fledged per nest from the last five years are considered together in an analysis of variance (Table 15), we detect no significant effects due to plot or plot/year interaction. There is a highly significant effect of year (F=22.51, P<0.0001). This effect is primarily due to the episodes of inclement weather in 1986 and 1989 which severely limited the numbers of young fledged from most nests.

Landmark growth events. The mean number of days to eye opening in 1989 (Table 16) was longer at the Pirlot Road test plot (8.6 days) than at

Tachycineta Meadows control (7.8 days), however these differences were not significant in analysis of variance (Table 17, P=0.166). Eyes opened much earlier in 1986 on both the test and control when compared to 1987,1988 and 1989 (Table 16). In all years, there is a significant effect of nest on the age at eye opening (P<.01). The scoring of eyes closed or open in the field is somewhat subjective and may be biased depending upon observer, lighting conditions and other factors. In addition, we only observe the young on an every-other-day basis. The resulting increase in the variance further reduces our ability to detect subtle differences in age of eye opening. We will continue to score the age of eye opening, but with increased attention to problems in assessing the status of the eye.

Mean number of days to feather eruption in 1989 was greater at the control (9.6 days) than at the test (9.1 days) but not significantly so (Table 18, P>0.57). No significant effects of plot were noted for 1986 or 1987 as well (Table 18). Like age at eye opening, in all years there is a significant effect of nest on the age at feather eruption (P=.0001). Contrasting feather eruption with eye opening, the eruption of primary feathers is generally a less variable measure than eye opening and is much less subjective in the field when the actual scoring takes place. We therefore have more confidence in using this variable as an assessment of ELF effects on developmental landmarks.

Statistical sufficiency of fecundity measures. We have examined the statistical power of test and minimum detectable difference for the measures of fecundity discussed above (Table 19, 20). We are currently able to detect changes of less than 10% of the mean for the variables measured, but the power of these tests are very low. If we set the power of the test at 70% certainty, we are still able to detect differences of less than 10% for clutch size and

hatch success, but only about 27% for fledging success (Table 19). Minimum detectable differences are larger for eye opening and feather eruption, but still are all below about 16%, with power again less than 30% (Table 20). With the power set to 70%, minimum detectable differences increase to mostly less than 45%, depending on the year (Table 20). We will therefore be less confident of rejecting the hypothesis of no difference in plots for these variables.

Mortality. Exposure data for nests, eggs, and nestlings used to assess mortality rates were calculated using the Mayfield method (Mayfield 1961, 1975). Units of exposure are egg days, nestling days, and nest days. For example, one nest with five eggs observed for four days would represent 20 egg days and four nest days of exposure. Data presented here include information from all active nests from all plots (five test plots pooled, two control plots pooled) and represent an overall nesting success analysis (Tables 21-25).

Egg mortality in 1989 was slightly higher on the test plots (2.20%) than on the control plots (1.87%). These small differences were not significant (G=3.11, df=1, P>0.05). Nestling mortality was also slightly higher on the test plots (6.25%) when compared to controls (5.81%), These were not significant differences (G=0.72, df=1, P>0.1). The likelihood of nest failure at anytime during the nesting cycle was also slightly higher on the test plots (2.57%) than the controls (22.2%), but not significantly so (G=1.00, df=1, P>0.1). Nest failure in 1989 occurred more frequently during the nestling phase of nesting due to the inclement weather, but the likelihood to fail was the same for both test and control during the incubation phase (G=2.05, df=1, P>0.1) and the nestling phase (G=0.09, df=1,P>0.5).

Overall, mortality of eggs and nestlings, and failures of entire nests was not significantly different between test and control plots in 1989. In previous years there have always been significant differences between test and control in at least one of these five measures, for reasons we could not ascertain.

In 1989, 285 adults were captured; 161 (56.5%) were new individuals and 124 (43.5%) were returns which were banded by us during previous seasons. The number of returning adults in 1989 was greater than previous years; 33.8% in 1988, 12.3% in 1987, 29.7% in 1986 and 16.6% in 1985. As many young as possible are banded before fledging; in 1989, 439 young were banded in the nest, 37% less than in 1988. In 1989, as in 1986, nest abandonment by the adults and the high mortality of young, caused by inclement weather, reduced the number of birds available for banding. The low number of returning birds in 1987 during nesting may be a reflection of the 1986 cold weather.

Growth. Curve fitting to growth data for individual birds during 1989 for body mass, tarsus and ulna growth was accomplished using the logistic model while wing growth was fit by the exponential model. These models produce the highest R^2 values, on average, compared to other growth models (see Ricklefs 1983, and Zach and Mayoh 1982, for discussion of various models).

The linearized form of the logistic model (Ricklefs 1983) was used to produce values for the growth rate constant and the inflection point for body mass, tarsus and ulna growth. Log transformed data from wing growth were used in an exponential model. The growth and inflection point variables for each nestling were included in the data set if there was a significant correlation between the variable and age. The data were then analyzed using nested

analysis of variance (NANOVA), with the effect of nests included within plots. Thus, the model may be written as:

$$Y_{ijk} = u + a_i + B_{ij} + e_{ijk}$$

where Y_{ijk} is the kth observation in the jth subgroup of the ith group, u is the parametric mean of the population, a_i is the fixed effect of the ith group (plots), B_j is the random contribution of the jth subgroup (nests) and e_{ijk} is the error term. A nested model was used to account for the known effect of parents on the growth of their nestlings. Ricklefs and Peters (1981) studying the European starling (Sturnus vulgaris) in Pennsylvania found the most significant contribution of variance to total variance in growth was due to the parents rather than variation in individual nestling growth or inherited growth traits. Our data on tree swallows shows similar partitioning of the variance in growth. The appropriate ratio for testing for a treatment (plot) effect is the mean square due to plot with the mean square due to nests within plots rather than the error mean square. This reduces the effective sample N to the number of nests, rather than the number of nestlings, and has some important impacts on the power of the test. This will be discussed in detail below after summarizing the findings for 1989.

In general, growth rates and inflection points (the intercept was not used in the analysis because its meaning from a biological point of view is not clear) were most strongly affected by nests within plots and least by plot (Tables 26 - 30). For weight, ulna and wing growth constants, no significant plot effects were detected in 1989 or in previous years.

Growth constant for tarsus, however, was significantly higher on the test plot compared to the control (Table 28 for NANOVA, Table 33 for means). Growth constants for tarsus have varied over the years from .22 to .35 (1985) (Table 33) but none of the differences between plots have been significant.

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In 1989, we used a different protocol for selecting the asymptotic value for

tarsus that involved deleting all measures beyond the age where the asymptote

was reached. This seems to have significantly reduced the variability in the

data for tarsus (compare coefficients of variation for earlier years with

1989). Until we submit all previous years data to the same procedure, we can

not conclude that we see a plot effect due to the antenna operation in 1989.

Earlier years show as large or larger differences in the means between test

and control plots (Table 33).

For weight, tarsus and ulna inflection points (wing growth model does not

have an inflection point), there was no plot effect in 1989 or previous years.

However, for all variables, except for ulna growth in 1987 and 1986 (Table

30), and tarsus growth in 1988, a highly significant effect was found for

nests within plots. Thus, nests differ greatly between themselves, but not

between plots (Tables 33 and 34), for the measured variables. We do not

currently understand why nests showed no significant variation in ulna growth

in 1986 and 1987 but did so in 1985, nor why tarsus growth showed no

difference in 1988 but did so in previous years.

We have examined the power of each performed test and the difference in

means that can be detected with our current data (Zar, 1984, p 260). The

results (Tables 35, 36) indicate that we are able to detect differences in

test versus control means of less than 10% in most variables for growth and

inflection point, which is half of our stated detectable difference. The main

exception is for tarsus growth in 1986 and for tarsus inflection point in all

years. However, the power of the performed tests is less than 30% for all

years and variables. With the power of the test set for 70%, the minimum

detectable difference, as a percentage of the mean, for growth constants

(Table 35) varies from a low of about 12% for wing growth and a high of 59%

for tarsus growth. The results are comparable for inflection point (Table 36) but averages above 85% for tarsus for all years.

This analysis clearly points to problems with the measurement of the tarsus growth. Examination of raw data on tarsus growth shows there is more variation in the measured length on the same individual from day to day than for other variables. Tarsus length reaches an asymptote earlier than other body parts we measure. The measures after final length is reached are variable, apparently reflecting observer variation in measurement. In 1989, we deleted measures taken after the asymptote was reached, and this reduced the minimum detectable difference and increased the power of the test for both measures of growth and inflection point over earlier years (Tables 35 and 36). We plan to make this modification to earlier years' data and see if the variation there is also reduced.

The analysis of power and detectable difference allows a more detailed examination of the method of analysis we are using for tree swallow growth. One striking feature of the growth data fitted to the logistic model (or any of the other growth models) is that the coefficient of variation is higher, by about 10%, for all variables compared to the raw data itself. Thus, the fitting procedure is introducing additional, undesirable variation into the data, a finding reported by Zach (1988) as well. We are now ready to use a procedure for fitting the data directly using procedure NONLIN in SYSTAT (Wilkinson 1988). This procedure uses a least squares algorithm to fit user specified models to data. In our case, we will specify the logistic equation and estimate the asymptote, hatch value and growth constant directly. This procedure is recommended by Ricklefs (1983) as superior to the linearized logistic because one does not have to deal with selecting the asymptotic value from the data, a process that is crude and probably responsible for most of

the additional variance we see in our fitted data. We will also use raw data
in procedures such as Repeated Measures ANOVA for comparison with the model
fitting procedure.

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PARENTAL AND NESTLING BEHAVIOR, AND FECUNDITY, GROWTH, AND MATURATION STUDIES - DEERMICE

I. Purpose

The purpose of these studies is to characterize several aspects of the reproductive process in deermice at test and control sites and to test for possible effects of the ELF Communication System on these variables. Specifically, the rates of growth and development of nestlings are compared between test and control sites and for each site from year to year. All of these work elements are described together in this one section because they are all performed on the same families of mice.

II. Methods

These studies were conducted within enclosures because free-ranging mice have been found not to remain resident in nest boxes for long enough periods for us to obtain the data desired. The enclosures are large: 6.1 by 5.8 m. Ten enclosures have been constructed within mixed deciduous forests at both the test and control plots. They are open at the top to allow free passage of atmospheric electromagnetic fields and free exposure to weather. Furthermore, they were constructed primarily of acrylic plastic sheeting, which is permeable to atmospheric electric fields according to IITRI engineers. Briefly, the walls of the enclosures consist of acrylic sheeting attached to cedar posts extending about 60 cm above ground and projecting about 15 cm below ground to prevent mice from digging out. A 51-cm-wide sheet of acrylic placed horizontally along the top of each wall prevented animals from climbing over the wall. Tree trunks were sheathed with sheets of high-density polyethylene to prevent mice from climbing in or out of the enclosures via the trees. Each enclosure was provided with a nest box and a feeding and watering station. The nest box can be opened to permit access to the mice.

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Small enclosures (termed holding facilities or "hotels") built according to the same design, but measuring just 1.2 by 1.2 m, were also constructed at the same sites. These enclosures were used as holding facilities for mice awaiting study in the large enclosures. The mice to be studied were captured in mixed deciduous forest near the enclosure sites. They were set up as male-female pairs. Later the females were transferred into the large enclosures when visibly pregnant. They gave birth in the enclosures and reared their young to the age of weaning.

Newborn young were toe-clipped for identification when 4 days old. From then until they were 22 days old, their growth was followed by weighing every other day to an accuracy of 0.1 g using a Pesola scale. Initial litter size and subsequent deaths were recorded. The age of eye-opening and incisor eruption was recorded as an index of developmental rate.

III. Results - 1989

The growth and development of 3 litters from 3 females at Pirlot test plot and 3 litters from 3 females at Michigamme control plot were monitored during 1989. Predation by raccoons, skunks and bears of hotel and enclosure mice was considerable on both test and control plots this year. Of the 10 females which dropped litters at Pirlot Road test plot 7 were predated. At the Michigamme control plot 7 females produced young, however 4 were predated. Efforts to trap predators met with limited success. In subsequent years, we plan to set predator traps at the beginning of the season. As another precautionary measure, we have bolted the enclosure nestboxes to posts to prevent the nestboxes from being turned over.

Growth of Young. A perusal of the growth in body mass of nestlings indicates that growth curves often appear non-linear. Although littermates consistently exhibit similarly shaped growth curves, there are apparent

differences in curves among litters of different females as well as differences between litters of the same female (i.e., some are exponential, some sigmoidal, etc.). While this variability in the shape of growth curves among (but not within litters) is interesting, it precludes the use of any particular non-linear model (e.g., logistic growth model) to estimate and compare growth rates in these mice. Therefore, growth rates have been estimated using linear regression analyses for growth of each individual up to the time of weight recession which appears to be correlated with weaning (Table 37). Nested ANOVA of growth rate due to mothers nested with plot yielded a significant effect of mother but none due to plot for 1989, 1988 and 1987 (Table 38). At this writing, we do not have any hypotheses as to the nature of the mother effect, although we now suspect that the number of littermates may be of considerable importance, based on our preliminary analyses of the effects of number of young on growth rates in tree swallows.

The power of the test and the detectable differences were estimated for each year from 1986 to the present (Table 39). The minimum detectable difference ranged from about 11% in 1986 to a high of about 75% in 1987. Minimum detectable differences at 70% power are very large and variable from year to year, much more so than growth for tree swallows. Perhaps this reflects problems in field measurement, but we think it is more a function of the response of the deermice to captivity and handling. They are much more sensitive to handling than the birds.

Age at eye opening and incisor eruption in deermice were similar between plots in 1989 and other years (Table 40). Age at eye opening was not significantly different between plots in 1989 (Table 41) as was the case with incisor eruption (Table 42) with the only significant effect being that of mother.

Power of the test for eye opening and incisor eruption are less than or equal to 30% and minimum detectable differences range from about 7% to 34% (Table 43). At 70% power, minimum detectable differences vary from about 32% to over 167%. We therefore have relatively poor ability to see small changes in these variables that may result from EM fields generated by the antenna.

Much of the variation in growth and maturation of young mice can be attributed to the frequency of visits we make to obtain the data (every other day) and also the apparently inherent response to disturbance caused while obtaining measurements. Thus an animal categorized as not having eyes open on a particular day will not be checked again for two days. This produces a built in error of two days. Thus, we do not feel we can obtain fine enough resolution for these variables to meet our statistical criteria without increasing the frequency of visits, yet it is also clear that handling is a major factor affecting growth of the nestlings. We are investigating the possibility of modifying our present work schedules to allow more frequent visits and also techniques that may minimize the effects of handling and disturbance.

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HOMING STUDIES - TREE SWALLOWS

I. Purpose

The purpose of these studies is to measure the homing success of tree swallows at test and control sites and to test for possible effects of the ELF Communication System on such success. Variables measured are the proportions of swallows that successfully return home after displacement and the time required for each bird to return home. Birds returning to their nestbox within 300 minutes are considered successful.

II. Methods

Adult birds were captured at the nest box using a passive nest box trapping device (Hussell, per comm). Captures took place between 0800 and 1100 to allow adequate feeding of the young in the nest prior to capture. Following capture, each bird was sexed (using the presence of a cloacal protuberance for males and brood patch for females) and aged using plumage characteristics (Hussell 1983a). Birds were banded using a standard U.S. Fish and Wildlife band and were color marked on the breast using "magic markers" to provide rapid and positive identification while in flight. Birds were placed in wire cages which were covered with black cloths, and then driven to the release sites.

In our first studies of swallow homing in 1984 and 1985, we released birds at all four cardinal compass directions (east, west, north, south) at test and control sites. The results revealed no differences in homing success from one compass direction to another. Furthermore, because tree swallows probably home without regard to habitats they fly over, and they are not likely to be exposed to any different hazards (predators, etc.) in homing from one direction as opposed to another, we feel justified in releasing birds at just one compass direction. Using just a single release point at test and control

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sites is more efficient in terms of personnel effort than use of four release points and thus permits adequate sample sizes to be obtained more expeditiously.

The release points are located in open areas that are at a distance of 30 km from the nest sites and at a compass direction 20 degrees NE of the nest sites (Figure 1). This distance was chosen because it is greater than the distance corresponding to a drop of two orders of magnitude of potential electromagnetic fields given off by the Communications System. The direction of the release points in relation to the nest sites was chosen so that birds attempting to return to the test site in a straight line will cross both east-west legs of the antenna configuration, areas that would supposedly be maximally influenced by ELF electromagnetic fields. Upon release, the time, vanishing vector, and weather conditions were noted. Observers located near the nest boxes recorded the time at which the birds return. Birds at each release site were released singly, with the subsequent bird released when the first had disappeared from sight (approximately 3 minutes).

III. Results - 1989

Data were collected at Panola Plains (PPC) and North Turner (NTT) and Cleveland Homestead (CHT) plots. Due to the inclement weather which caused a high rate of nest failure, only a small number of nests remained available for the homing study (Table 44 and 45). All of the displaced birds, with the exception of one at Panola Plains, returned to the nest within the 300 minute time limit. The small numbers preclude the use of any contingency table tests to determine differences between test and control.

Mean time to return was not different between the two test plots ($t=-0.28$, $P=0.78$), so they were pooled to compare to the control. Mean time to return on the test plots was significantly less than on the control plot (118 vs. 168

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minutes, t=2.65, P=0.013), which is a continuation of a trend which has persisted through the past four years of the study (Table 45). In addition, the return times in 1989 for both test and control were faster than previous years, particularly on the test plots. When return times for all four years are considered together in an analysis of variance (Table 46), a significant effect of plot is detected (F=34.46, P<0.0001), the return times being consistently faster on the test plots. A consistent difference in return times of approximately 50 minutes faster on the test plots has been maintained during 1987, 1988 and again in 1989. In addition, a significant effect of year is also shown (F=3.17, P=0.026), due to the much shorter return times in 1989 on both test and control. No plot/year interaction was detected.

Overall, analyses show that displaced birds from the test plots exhibit a higher likelihood to return (Table 47) as well as faster return times. The reason for the observed difference remains unexplained. We are concerned about the possible relationship of homing performance and antenna testing, which began in 1986 and continued through 1989 with increased power each year. We have found no unusual patterns of 76Hz or 60Hz EM fields measured at Panola Plains or the Panola Plains release site. In late June, 1988, personnel from IITRI followed the return path of the control birds from the release point to Panola Plains in a light aircraft. Their findings showed no unusual generators of EM fields along the flight path other than the distribution lines along Highway M-69 and the Wisconsin Electric Way and Hemlock power dams (Figure 4). These dams had been previously brought to our attention by IITRI, and data on their operation was obtained. During the seven days of homing in 1986 there was no power being generated by these dams. There was power generation for two of the five days during which we performed our studies in 1987, yet statistical tests reveal no differences in likelihood to return or

return times on those days when compared to the other three days of homing on the control plot. Thus, at this writing, we do not have any idea what is causing the observed differences between times to return for test and control birds.

Power of the test of return time yields a detectable difference of about 25% at a power of greater than 99% (Table 48). If we apply our relaxed standard of 70% power, then differences as small as 11% in returns should be detectable using our current sample sizes and research protocol.

HOMING STUDIES - SMALL MAMMALS

I. Purpose

The purpose of these studies is to measure the homing success of small mammals at test and control sites and to test for possible effects of the ELF Communication System on such success. Variables measured are the proportions of individuals that successfully return home after displacement and the time required for each individual to return home. The principal species studied are deermice and chipmunks.

II. Methods

During our initial studies on mammal homing in 1985 (Beaver, et al. 1986), we displaced chipmunks and deermice in all four cardinal directions in order to investigate any directional biases in homing ability. No such biases were found even though animals displaced west and north on the control and test plots had to cross the sham corridor or actual antenna corridor, as well as somewhat different habitat types. However, our sample sizes were small for any particular displacement direction (maximum of 10 animals) and we therefore could not be certain of the robustness of our tests. Thus, in contrast to the work on swallow homing, we decided to reduce the number of displacement directions to two rather than one. Reducing the number of directions from four to two increases efficiency of sampling. By using two directions rather than one, however, we maintained the diversity of habitats and corridor crossings at each site, thus helping to insure that we are further able to examine the effects of habitat conditions as well as potential effects of ELF on homing behavior.

The small mammal homing study was conducted on two trapping grids, one at the Pirlot road test site and the other at the Michigamme control site. Due

to the low chipmunk and deermouse populations noted in 1985 and 1986, the size of the trapping grid was increased in 1987. Each grid contained 100 stations spaced 15 meters apart rather than ten meters, therefore increasing the area covered to 1.8 ha versus 0.81 ha as before. One Leathers live-trap was placed at each station, baited with peanut butter and rolled oats. The grids were situated on the east side of both the ELF ROW and the sham ROW. A habitat buffer between each ROW and its respective trapping grid was increased in 1987 to 50 meters, rather than the 10 meters of 1985. This increase helped insure that both the grids and their displacement lines were located in more uniform habitat, a continuous mixed deciduous forest dominated by sugar maple (Acer saccharum).

Trapping began on 8 July and ended on 4 August, 1989. Traps were checked twice daily (ca. 0800 and 1700) and rebaited as necessary. Because of the small sample sizes obtained for other species in 1985, only eastern chipmunks and woodland deermice were displaced in the following years. Each animal was weighed, sexed, and toe-clipped or ear-tagged for individual identification. Reproductive condition, station number, and capture time were also recorded. Individuals were kept for displacement after their third capture; such animals were deemed to be residents of the area where the trapping grid was established which, hopefully, insured their detection by continued recapture on the trapping grid upon returning from displacement. Before being displaced, each animal was kept in a laboratory cage supplied with nesting material, lab chow, and water. Cages were placed in screened-in storage sheds located near each site. Displacements took place during, or just prior to, the next activity period following capture; deermice (nocturnal) were displaced at dusk (ca. 1900) and chipmunks (diurnal) were displaced in the morning (ca. 0800). Each animal was displaced 450 m from the trap it was captured at when

kept for displacement. Displacements took place to the south and west of the home grids. The exact point of release was adjusted to reflect the point of capture on the home grid; this way all individuals were displaced exactly the same distance from their capture point. Trapping continued for five days after the last animal was displaced.

The displacements to the south were through continuous forest, whereas those to the west required returning animals to cross the antenna corridor at the test site and the sham corridor at the control site. Use of the two displacement directions thus specifically allowed us to test for directional differences in return rates which might occur due to the fact that animals returning from the west must pass beneath the antenna line, potentially the area of greatest electromagnetic disturbance.

III. Results - 1989

Overall, population numbers remained low at both test and control sites. The presumed reason was the presence of Tyzzer's disease in wild populations of deermice and chipmunks. We reported earlier that trappable populations of these species were lower during 1987 and 1988 than in 1985 and 1986 for the same presumed reason.

A total of 34 deermice (13 control, 21 test) and 39 chipmunks (16 control, 23 test) were displaced in 1989 (Table 49). Fewer deermice were displaced in 1989 (34) compared to 1988 (55), whereas chipmunk numbers increased from 22 displaced in 1988 to 39 in 1989. No differences in likelihood to return were detected between the two displacement directions, so the data were pooled. For chipmunks there were no differences in likelihood to return to the home area between test and control sites (G=0.3063, P>0.50) in 1989. When chipmunk displacement data are pooled for the years 1986-1989 (Table 49) we see a significant overall effect of plot and year (G=21.299, df=7, P<0.005). When

this table is broken down into its components of plots (Table 49, test and control pooled over years and compared), there is a significant effect due to plot (G=3.85, df=1, P<0.05); the likelihood to return being greater on the control plot. In addition, when only years are considered (Table 49, years pooled over test and control and compared), there is also a significant effect due to year (G=17.857, df=3, P<0.001). This appears to be due to overall rates of return being higher in 1989 and 1986 when compared to 1987 and 1988. Unfortunately, 1987 and 1988 were also years of lowest population numbers and subsequently low sample sizes.

For deermice in 1989, the likelihood to return was significantly greater on the test plot (Table 49, G=4.830, df=1, P<0.05). When deermouse displacement data are pooled for the years 1986-1989 the likelihood to return is independent of both plot and year (G=13.795, df=7, P>0.05).

It now appears that we have a parallel result for mammals with homing in tree swallows. Returns to the test plot are more likely for both chipmunks (all years) and deermice (1989 only). We do not at this time know what aspects of the antenna fields and/or characteristics of the plots are responsible for the observed differences. We are studying the data carefully and will continue to look for causes of the observed pattern.

SMALL MAMMALS AND NESTING BIRDS 1989 ANNUAL REPORT

DEVELOPMENTAL STUDIES

I. Purpose

The purpose of these studies is to characterize aspects of normal development in tree swallows and to investigate potential effects of ELF radiation on development. Specifically, early embryological development of tree swallows is being characterized, developmental abnormalities in field populations of tree swallows are being described and their incidence in test and control plots is being determined, and the sizes of eggs collected from test and control plots is being compared.

II. Methods

Tree swallow eggs were collected from nests during late May and early June. Entire clutches were removed from 15 nests in control plots TMC and PPC and 15 nests in test plots FST and FNT exposed to ELF irradiation. Nests were inspected daily and eggs were numbered as they were laid. Eggs were collected 4 days after the appearance of the last egg. A total of 160 eggs were inspected.

Collection of eggs and subsequent analyses were carried out by different investigators to avoid bias. Each nest was coded in such a way that the investigator carrying out the analysis was unaware of the test or control status of eggs until analysis was completed.

All eggs were weighed to two decimal places on a Sybron Digimetric balance and measurements of length and breadth obtained using vernier calipers. For fifteen eggs (clutches 28, 29, and 30) egg volumes were obtained by water displacement. This allowed the calculation of a constant, K, which was used to determine an approximation of egg volume from the linear measurements.

All embryos were dissected off the yolk into Howard Chick Ringers solution (Johnson and Volpe, 1973), analyzed briefly by microscopic observation and

fixed in either Bouin's solution for further observation and histological study or 2.5% glutaraldehyde in Howard Ringers for scanning electron microscopy.

Embryos placed in Bouin's were fixed for 24 hours and dehydrated through a graded series of ethanols. Picric acid from the Bouin's solution was removed with 70% ethanol saturated with Li_2CO_3. Embryos were stained as whole mounts with either an alcoholic carmine solution (Watterson and Schoenwolf, 1984) or an alcoholic eosin solution and cleared in methyl salicylate. All embryos were then observed carefully with an Olympus stereomicroscope using transmitted light and a photographic record of each was obtained. The use of methyl salicylate as clearing agent allows material to be observed as a whole mount and stored without undue tissue hardening. Subsequently, material can be embedded in paraffin and used for routine histology. Currently, normal embryos at all stages observed and abnormal embryos are being sectioned for histological examination.

Embryos that were placed in glutaraldehyde were fixed for 30 minutes, dehydrated in graded ethanols, critical point dried and sputter coated with gold. Observations were made on a JOEL 35CF (15 kV) scanning electron microscope.

All embryos were staged using the chick embryo series of Hamburger and Hamilton (1951) as a reference. Abnormalities were tabulated and characterized as completely as possible.

III. Results - 1989

Normal development. The early embryology of tree swallows closely parallels that of the chick as described by Hamburger and Hamilton (1951). By 4 days incubation, many embryos had reached Stage 23, a few, stage 25. As reported previously (Beaver, Hill and Asher, 1986), an asynchrony of

development was observed with the last laid egg routinely lagging several stages behind the others. This asynchrony probably results from the nesting behavior of females. In the present study, females are frequently observed to spend time on the nest prior to laying the last egg. Such behavior is not restricted to tree swallows, but is common among small, altricial passerines (Clark and Wilson, 1985). The degree of developmental asynchrony, then, will depend upon the extent of incubation prior to clutch completion.

Normal embryos, some viewed with the scanning electron microscope, others viewed after staining as whole mounts, are shown in Plates 1 and 3. Although some differences between chick and tree swallow development have been observed, the close similarity between the two species is apparent.

Abnormal development. Development of the following morphological structures was checked for each embryo - head (including brain, eye and ear), heart, branchial arches, spinal cord and somites, limb buds, allantois, amnion and the flexion and rotation of the embryo. The types and frequencies of abnormalities are shown in Table 50. Two test eggs and one control egg showed no or very little development. One embryo from a test plot underwent a reverse rotation so that the head and heart p.inted to the left instead of to the right. One, again from a test plot, was completely abnormal, the only recognizable organ being the developing ventricle, which was evaginated (Plate 4, No. 5). The most common abnormalities involved the spinal cord. Three embryos from control plots and three from test plots had spinal cords that appeared wavy instead of straight or curved, or were S-shaped or too short. A second type of spinal abnormality resulted in embryos which had backs that appear "dented". Seven of these embryos were detected, four in a test plot (Plate 4, No. 1-4) and 3 in a control plot (Plate 2, No. 1-3). This particular abnormality shows a very strong "nest effect," since all the

affected embryos came from only 2 nests. Since one nest was from a control plot and one frcm a test plot, there is currently no reason to think that ELF radiation is a causative agent. The "dented back" abnormality seems to be associated with the rotation of the embryo. During rotation, embryos often have a rather irregular dorsal outline but this smoothes out as rotation is completed. Each of the above two clutches contained embryos showing dented backs, in which rotation should have been completed, and embryos at a much earlier stage of rotation in which the problem could not be detected. "Dented back" embryos from 1 clutch were fixed in glutaraldehyde and have been observed with a scanning electron microscope (Plate 2). At higher magnifications (Plate 2, No. 3 and 4), a comparison of the "dented" region with a similar area from a normal embryo shows a folding of the back.

A condition which may be similar has been reported by Wyttenbach and Hwang (1984) and Garrison and Wyttenbach (1985). These investigators found that chick embryos exposed to organophosphate insecticides develop wry neck, short neck and other cervical defects including vertebral fusion. They conclude that the primary effect of the insecticide is on the notochord which appears folded in histological sections. The tree swallow embryos shown in Plate 2, No 1-4) are now being sectioned and will be compared with the chick material.

Finally, 4 embryos showed an abnormal development of a structure which is probably the allantois. During normal development, the allantois becomes visible at Stage 18, first as a solid structure, then as a vesicular one, pointing to the right, and thus the ventral side of the embryo. In these 4 embryos (and in 2 others which were eliminated from the study because the egg shell was dented by observers or the parent birds in the nest) a thickened projection points to the left, or dorsal side of the embryo (Plate 4, No. 6). All embryos in which this was observed were Stage 18 or 19. Since no older

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embryos showing a dorsally directed allantois occurred, this difference may correct itself with subsequent development.

Frequency of abnormalities. To determine the significance of the frequency of abnormalities, Chi square analyses were carried out in normal and abnormal embryos in test versus control plots. Because the determination of "abnormal" is not clear-cut, tests were carried out in different ways. Table 51, a contingency table, shows the analysis of embryos excluding the 4 with the allantois directed dorsally. Table 52 shows the analysis when these embryos are included. Table 53 is an analysis excluding both embryos with dorsally-directed allantois and "dented" backs. In all cases, very low values for X^2 (0.63, 0.97 and 0.96 respectively) were obtained, indicating that there is no difference in the frequency of the abnormalities detected in test and control plots.

As reported previously, the overall incidence of abnormalities is quite high (Beaver, Hill and Asher, previous reports). We find that approximately 11% of the embryos inspected in 1989 are abnormal. This value assumes that embryos showing the posteriorly directed allantois will develop normally. If these embryos are included, the incidence rises to 14%. These values are in good agreement with results reported elsewhere in this report. In test and control nest boxes in which hatching success was monitored, approximately 17% of the eggs failed to hatch (this report, p.24). This high rate of failure to hatch is not unique to the tree swallow population under study. Paynter (1954) found a 15% hatching failure in tree swallows on Kent Island, New Brunswick and Chapman (1955) found an average failure to hatch of 20.5% in a 12 year study of a single tree swallow colony in Princeton, New Jersey.

Size of eggs. Since avian embryos must develop in a closed system, the resources allocated to each offspring during oogenesis could have a marked

influence in determining chick survival. If females forage less effectively

in some situations than others, eggs may be of lower nutrient value and chick

survival compromised. To determine whether ELF radiation affects the amount

of nutrient deposited in eggs, each egg at the time of collection was weighed

and measurements of length (L) and breadth (B) obtained using vernier

calipers. By making the assumption that the volume of a tree swallow egg

varies with the constant, K, the following formula was obtained:

$$V_{egg} = K\pi(B/2)^2(L/2)$$

or more simply:

$$V_{egg} = K_v B^2 L$$

The volumes of fifteen eggs (nests 28, 29, 30) were measured by water

displacement. Using these values, a value of $K_v = 0.50$ was obtained. This K

value is in good agreement with that determined for other species. Hoyt

(1979) reports K values with a mean of 0.507 ± 0.007 for 26 avian species.

Using $K_v = 0.50$, volumes of 160 eggs from test and control plots were calculated

for each clutch are shown in Table 54. Egg volumes from test and control

plots were compared using a nested ANOVA (Table 55). No significant

difference between the two groups was found.

Calculated volumes of eggs are quite similar to weights. We have

estimated size by volume rather than by weight because evaporative water loss

through the shell is a continuous process. As a result, the first egg laid

has lost considerably more weight than the last by the time the eggs are

collected.

Comparisons with other species. The development of the domestic chick is

the standard against which most other species are measured. Early tree

swallow development closely parallels chick development; however, some

differences become apparent as early as Stage 10. Apparent differences

include the constriction of the eye stalk, the timing of pigmentation of the eye, the shape of the jaws as they form, the shape of the developing mesencephalon and the relative size of wing buds as compared to leg buds. These morphological features will be studied in histological sections as well as whole mounts and compared with those of the chick. Most of the differences are slight. One difference that is quite apparent concerns the formation of limb buds. In the chick, which even as an adult rarely flies and mostly runs, development of the hind limb precedes that of the forelimb (Hamburger and Hamilton, 1951). In tree swallows, this order is clearly reversed, with wing buds enlarging more rapidly than leg buds (Plates 1 and 3).

The bulk of the information available on avian embryology has been obtained using chick and Japanese quail (Hamburger and Hamilton, 1951; Freeman and Vince, 1974). A few studies have been carried out on gulls, kittiwakes and guillemots (Haycock and Threlfall, 1975; Maunder and Threlfall, 1972; Mahoney and Threlfall, 1981). All of these species are precocial. A dearth of information exists concerning the embryological development of altricial, passerine species. Consequently, information concerning development in this important group of birds will be of significance.

SMALL MAMMALS AND NESTING BIRDS 1989 ANNUAL REPORT
STUDIES OF MAXIMUM AEROBIC METABOLISM

I. Purpose

The purpose of these studies is to measure the peak aerobic metabolism of animals during winter at test and control sites and to test for possible effects of the ELF Communication System on peak metabolism. The principal species studied are chickadees and deermice.

II. Methods

Collection and care of birds. To attract chickadees for study, feeding stations were established in December and kept stocked throughout the winter with sunflower seeds. Chickadees were mist netted as needed from these stations. Upon capture, birds were weighed to the nearest 0.1 g using a Pesola spring scale and marked with a colored plastic leg band for individual identification. When released from captivity, they were banded using a standard U.S. Fish and Wildlife Service band for permanent marking. Birds were housed singly in wire mesh cages (28 x 18 x 31 cm). Shelled sunflower seeds and snow or water were available ad libitum. In addition, each morning and late afternoon, meal worms were provided in excess. The cages were kept in a screened outdoor holding facility, which provided natural lighting and temperature conditions.

Collection and care of mammals. Trap shelters were established in late November, prior to any substantial snowfall. The shelters were located along wandering lines situated approximately 75-250 m from the antenna or sham corridor. The habitat was northern hardwoods dominated by maple, basswood, and elm, typical of the area. Each shelter was a plastic waste container placed upside-down on top of the ground layer, with a covered top opening which provided the researcher access to the ground layer once snow was present. Mice entered the shelters through the interface between the ground

layer and the wall of the shelter. One Leathers live trap was placed .n the bottom of the shelter and baited with rolled oats, peanut butter, and sunflower seeds. Polyester batting was provided in the trap for nesting material. Traps were prebaited and left open one month prior to actual trapping to insure that small mammals would include the stations in their subnivean runways. Researcher travel on the sites was by snowshoe along a single trail to minimize disturbance of the subnivean air spaces which are critical to small mammal movements.

Trapping was begun at the start of January and continued intermittently, according to need for animals, through March. Work was focused primarily on the deermouse. Upon capture, individuals were toe-clipped for identification, sexed and weighed to the nearest 0.1 g with a Pesola spring scale. Once at the lab, animals were transferred to standard plastic lab cages (29 x 18 x 13 cm) with wire lids and provided with wood shavings, polyester batting, and a diet of sunflower seeds, lab chow, and apple and snow for moisture. Cages were housed in an open outdoor facility which provided natural lighting and temperature conditions.

Laboratory methods. To elicit a peak rate of oxygen consumption, we used a refined version of the helium-oxygen (helox) method first introduced to the study of small-animal physiology by Rosenmann and Morrison (1974). Placing an animal in a helium-oxygen atmosphere at a given ambient temperature greatly increases the individual's rate of heat loss by comparison to the rate in air (mostly nitrogen-oxygen), due to the relatively much higher thermal conductivity of helox. Thus, the animal must produce heat more rapidly in helox than air if it is to maintain a stable body temperature.

Whether the rate of oxygen consumption measured in helox is in fact a true peak metabolic rate depends partly upon the ambient temperature. Identifying

the true peak for an individual therefore entails studying the animal at a series of ambient temperatures. Specifically, study at a minimum of three ambient temperatures is required for a definitive determination: there should be a measurement at the temperature that elicits the peak, and also there should be measurements at temperatures higher and lower, demonstrating that the rate of oxygen consumption in helox falls off if the temperature is either raised or lowered from that eliciting the peak. Of course, the temperatures of interest are unknown at the onset of work on an individual. Thus, in principle, many measurements would have to be made on an individual before its peak would be definitively identified. In practice, experience often permits us to know in advance the temperature at which the peak will occur. Therefore, we often need to test an animal at just three temperatures to establish its peak definitively. The spacing we have used between temperatures is 5°C. Thus, if we test an animal in helox at three ambient temperatures that are 5°C apart (e.g. -10, -5, 0°C) and if the highest measured rate of oxygen consumption occurs at the middle temperature, we conclude that we have identified the animal's peak rate definitively.

Tests were not carried out on the day of capture to reduce any effect of capture stress. To further avoid adverse effects of stress, animals were tested only once on any given day.

Prior to a test animals were weighed to the nearest 0.1 g on an Ohaus triple-beam balance, and their body temperature (T_b) was measured by inserting a copper-constantan thermocouple probe 2-3 cm colonically. Then each animal was placed into a metabolic chamber. Chambers were constructed from new one-half gallon paint cans, with inflow and outflow ports in the lid. The inside surfaces were painted with 3M ECP-2200, for an emissivity of nearly 1.0. A 0.5-inch-mesh hardware cloth floor covered with Dip-It plastic coating was

used to elevate the animal above the bottom of the can, thus helping to insure proper airflow around the animal and permitting urine and feces to drop away so as not to wet the animal. The outflow port of each chamber houses a 36-gauge copper-constantan thermocouple to monitor chamber temperature, which is maintained by immersion of the can in a Forma Scientific 2325 water bath using ethanol as antifreeze. All temperature probes are connected to a Leeds and Northrup 250 Series Multipoint recorder which can be read to the nearest 0.1°C.

Measurements were carried out during daylight hours. Food was provided during measurements. Specifically, apple was provided for the mammals, and shelled sunflower seeds and a mealworm were provided for the chickadees. The metabolism chambers for the birds were equipped with a small light that provided dim illumination; without this light, the chickadees (which are diurnal feeders) would not eat. Our decision to provide food during tests is based on extensive preliminary experimentation and is predicated on the following considerations: (1) Animals in nature are able to feed during the day; the birds are diurnal foragers, and the mammals can feed from caches. (2) In the mice, the variance in results is lower when food is provided than when it is denied. (3) In the birds, there is evidence that fasting during these types of experiments increases the probability of death.

Oxygen consumption was measured using an open-flow system. Briefly, gas (air or helox) was pumped through the metabolic chamber at a measured flow rate, and the reduction in its oxygen content was measured. From these data, the rate of oxygen use of the animal could be calculated. The oxygen content of gases was measured with an Applied Electrochemistry S3A oxygen analyzer and recorded on a Houston Superscribe potentiometric recorder. Gas flow rates were measured with Brooks 1110 rotameters. The rate of oxygen consumption was

calculated according to the formulas in Hill (1972a, method B), taking cognizance of the mathematical relationship between gas composition and the output of the S3A analyzer. We have empirically verified that the S3A analyzer reads oxygen levels in helox with the same accuracy as in air.

Animals were provided with air during an initial adjustment period (0.7-1.5 hr) and then switched to helox. Flow rates were 600 ml/min in air and 900 ml/min in helox. The adjustment period in air was terminated once the metabolic rate remained approximately stable for 15 to 20 minutes. Upon switching to helox, a rapid transition to the new gas was made by purging the metabolic chamber at a rate of 5 liters/min for two minutes. Then the rate of flow was reduced to the 900 ml/min already mentioned. The maximal rate of oxygen consumption under the test conditions was generally achieved within 15-20 minutes after the switch to helox, and animals were rarely exposed to helox for more than 25 minutes. Following the measurement in helox, animals were quickly removed from the metabolic chamber, and a final T_b and weight were recorded. All thermocouples have been calibrated against thermometers whose calibration is traceable to the National Bureau of Standards. Flowmeters have been calibrated against a Brooks Volumeter also having a NBS-traceable calibration.

The one aspect of the measurement procedure that is open to significant subjective judgment is the determination of the particular time interval over which the maximum oxygen consumption occurred in each experiment. Because of the subjectivity involved in this determination, a "blind" procedure will be used once the Communication System antenna has been turned on and high-resolution comparisons of test and control sites are being carried out. The relevant raw data, as earlier noted, are recorded using a potentiometric recorder. These records are not marked as to the origin of the animals (test

or control site) but instead are identified simply by arbitrary, randomly assigned numbers. The final and definitive reading of the records will be carried out by a person who knows only these arbitrary numbers.

III. Results - 1989

Measures of peak metabolic rate were obtained on 17 deermice and 24 chickadees in the winter of 1989. All these measures were obtained within the first week after capture. Studies of animals in long-term captivity were not carried out in 1989 because an adequate data set on long-term captivity had been acquired at the end of the 1988 season (see later).

The data were analyzed as specified in the 1988 annual report. It will be recalled that measures of peak metabolic rate are assigned to 10 quality rating classes. Classes 1, 2, 3, and 4 represent peak determinations of highest quality. Classes 0 and 5-9 represent peak determinations rated as acceptable but nonideal. Statistical comparisons of sets of peak metabolic rates have been carried out using an analysis of covariance design unless otherwise specified. The logarithm of whole-body peak metabolic rate has been used as the dependent variable, and the logarithm of body weight has been used as the covariate. The reasons for the use of analysis of covariance and those for performing the analysis in the logarithmic domain are detailed in the 1988 annual report. Normality of the logarithmically transformed data for peak metabolic rates and body weights was assessed using probit plots, and homogeneity of variances was evaluated with Bartlett's test. Both normality and homogeneity of variances were found to be acceptable in all analyses.

Analysis of peak metabolic rates of deermice. The first step in this analysis was to determine if a difference existed between measures of peak metabolic rate that were rated in quality classes 1-4 (primary quality) and measures that were rated in the other quality classes (secondary quality).

This was done by pooling all data from both test and control plots into an analysis of covariance with a single factor: primary versus secondary quality rating. As in the past (see Beaver et al. 1988), the difference between the quality rating categories for deermice proved nonsignificant (P = 0.54). Thus, for analysis of plot effects, all peaks were pooled regardless of their quality rating. A single-factor analysis of covariance was performed on these pooled peaks, the factor being plot (test versus control). The effect of the covariate (body weight) was highly significant (P < 0.001). However, there was no significant difference between test and control plots (P = 0.27). Summary statistics are given in Table 56. We conclude that, for the deermice, peak metabolic rates measured in the first week after capture did not differ between test and control plots in 1989.

Analysis of peak metabolic rates of chickadees. The first step in the analysis was again to determine if a difference existed between measures of peak metabolic rate that were rated in quality classes 1-4 (primary quality) and measures that were rated in the other quality classes (secondary quality). This was done, as for deermice, by pooling all data from both plots into an analysis of covariance with a single factor: primary versus secondary quality rating. As in the past (see 1988 annual report), the difference between the quality rating categories for chickadees proved to be significant (P = 0.037), with the peaks of primary quality [mean = 25.0 ml O_2/(g X hr), N = 18] being higher than those of secondary quality [mean = 23.1 ml O_2/(g X hr), N = 6]. This result indicated that the peaks of primary and secondary quality should not be pooled. Thus, for analysis of plot effects, an analysis of covariance was performed on the peaks of primary quality alone. The effect of the covariate (body weight) was significant (P = 0.044). There was no significant difference between plots (P = 0.60), however. Summary statistics are given in

Table 56. For interest's sake, we have also carried out an analysis of covariance on all the peaks for chickadees, regardless of quality class. This analysis also indicated no difference between plots (P = 0.24). We conclude that, for the chickadees, peak metabolic rates measured in the first week after capture did not differ between test and control plots in 1989.

Minimal sample sizes. In Table 57, data on all peak metabolic rates obtained in the course of this research are summarized. The table includes the 120 measures summarized in Table 24d of the 1988 annual report plus the additional 41 measures obtained during 1989 and 30 measures obtained during 1985 (Table 24d of the 1988 annual report included data only from 1986, 1987, and 1988). Table 57 shows minimal sample sizes required to meet our stated minimal standards of statistical sufficiency (detection of a 20% difference between test and control plots at the 5% level of significance with 90% certainty). These updated estimates of sample sizes are identical to those computed in the 1988 annual report.

Final analysis of captivity studies: Introduction. Our goal in our studies of peak metabolic rates has been to estimate the peak rates of animals free-living in nature. To do the measurements, however, we must hold the animals in captivity for a number of days after capture, and, in our original designing of our experiments, the question arose of whether this time in captivity might alter the animals' peak metabolic rates. Specifically, we were concerned that peak metabolic capacity might fall in captivity because (1) with abundant food easily available, captive animals -- even though in outdoor cages -- need not expose themselves to the full brunt of outside conditions as much as wild animals in order to find sustenance and (2) captive animals are not able to exercise as much as wild ones, possibly leading to a

decrease in the mass or functional capacity of muscle, one of the important thermogenic tissues.

In outline, our experimental design for studies of whether peak metabolic rates decline in captivity has been as follows. We have captured animals and determined their peak metabolic rates as quickly as possible in the usual way. Then, we have continued to hold those animals in captivity (always in outdoor cages), and we have carried out measures of their metabolic responses in helox on one or two subsequent dates over the ensuing weeks. For each animal, these follow-up measures have been carried out at the ambient temperature that elicited the peak metabolic rate in the days immediately following capture. We have then compared the follow-up measures of peak metabolic rate with the initial measure of the peak to see if changes occurred.

Final analysis of captivity studies: Detailed methods. Forty deermice and 32 chickadees captured during January-March of 1985- 1988 were the subjects for these studies. All animals were housed in outdoor cages after capture. The cages for the deermice were plastic with wire tops, measuring 28 X 18 X 12 cm. Mice were caged singly and provided with wood shavings for bedding and polyester nesting material. Cages for the chickadees were constructed of welded wire and measured 30 X 20 X 25 cm. Each cage was provided with a wooden nest box measuring 8.5 X 8.5 X 9 cm. Wood shavings were provided as nesting material. The birds were housed in sets of 2-3 in 1985 and most of 1986 but were housed singly thereafter. All animals were provided with food and with snow or drinking water ad libitum. Food for the deermice was Wayne Rodent Blox with occasional apple, whereas that for the chickadees was shelled sunflower seeds and waxworms.

The initial determination of peak metabolic rate was started 1-2 days after capture. Our goal, as usual, was to test each animal at successive

ambient temperatures, 5°C (nominal) apart, until we determined an ambient temperature at which the measured metaboli   .te was higher than that at either the ..ext-higher or next-lower ambient temperature. The metabolic rate at that "middle" ambient temperature was then taken to be the animal's peak metabolic rate at capture. Table 58 presents data sets for two animals as examples. An animal was tested at only one ambient temperature per day. In most cases, only three runs were needed to determine the peak metabolic rate, and the determination was complete within 5 days after capture.

Following the initial de⁺ermination of peak metabolic rate, our goal was to retest each animal at about the same ambient temperature that initially elicited its peak at about 11-12 and 18 days after capture. The actual dates of retesting varied because of logistics, especially during the first two years of the study. Table 59 presents example data sets. Some animals were retested only once because they escaped, fattened excessively, or died before the date for their second retesting. Animals were dropped from experimentation if and when their body weight increased by 15% compared to their average weight during the initial determination of their peak metabolic rate. This weight criterion was adopted because, in animals that fatten, potential weight effects on peak metabolic rates become inextricably confounded with time-in-captivity effects on tissue metabolic capacities.

Final analysis of captivity studies: Results. The average weight of the deermice in the days just after capture was 18.7 g, whereas that of the chickadees was 11.6 g. Variation in weight among the deermice (SD = 2.6 g) was considerably greater than that among the chickadees (SD = 0.6 g). Table 60 summarizes the ambient temperatures at which peak metabolic rates occurred in the two species.

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The day on which we obtained the initial peak for each individual was determined serendipitously. For some animals, the ambient temperature used on the first test day proved to be the temperature that elicited the peak, and thus the peak was measured on the first test day. For other animals, the ambient temperature eliciting the peak was not used until the third test day or later. Table 61 presents the initial peak metabolic rates as a function of the number of days since capture when the peaks were measured. The metabolic rates are expressed in weight- specific terms in this table so as to adjust for differences in weight among individuals. Table 61 should not be misinterpreted as indicating that some individuals were tested five, six, or even eight times in order to determine their initial peak metabolic rates. Individuals were not necessarily tested each day. Table 62 summarizes the number of runs to which individuals were exposed in determining their initial peak metabolic rates.

The data in Table 61 can be used to appraise whether the peak metabolic rate changes during the first days in captivity. Within each species, we have compared the peaks that happened to be measured on days 1, 2, 3, 4, and 5 after capture to see if the magnitude of the peak metabolic rate is dependent on time in captivity during the first 5 days in captivity (peaks determined on days 6 or 8 were excluded from this analysis). The dependent variable was total (whole-body) peak rate of oxygen consumption. Body weight was entered as the covariate. Normality was assessed using probit plots. The analysis was carried out on log- transformed data for the dependent variable and covariate for two reasons: First, the approach to normality was better in the logarithmic domain than in the untransformed domain, and, second, the two variables are expected to have a linear relation in the logarithmic domain (see 1988 annual report) (covariate analysis removes linear effects of the

covariate on the dependent variable). Variances were homogeneous in the

logarithmic domain according to the Bartlett-Box test (P = 0.71 for deermice,

0.84 for chickadees). Homogeneity of the slopes of the covariate- dependent

variable regressions was tested by examining the interaction between the

covariate and factor (test day). The slopes were found to be homogeneous (P =

0.58 for deermice, 0.29 for chickadees).

Within each species, a significant portion of the variation in total peak

metabolic rate was attributable to the covariate, body weight (P < 0.001 for

deermice, P = 0.04 for chickadees). However, at a robust level, there were no

significant differences among days in the peak metabolic rates measured on

different days (P = 0.51 for deermice, 0.67 for chickadees).Thus, the data

support the conclusion that the peak metabolic rates of deermice and

chickadees do not change during the first 5 days in captivity. Because body

temperature potentially exerts important effects on peak metabolic rate, final

body temperatures are listed in Table 61 [note (e.g., Table 58) that

depression of the body temperature is typical of runs in helium-oxygen that

elicit peak or near-peak metabolic rates]. Differences in body temperature

between days were not significant (P = 0.06 for deermice according to analysis

of variance, P = 0.52 for chickadees according to a Kruskal-Wallis test which

was used because the data were not normal). Thus, body temperature is not a

confounding factor in interpreting the results on peak metabolic rate.

There is a concern as to whether runs in helium-oxygen may themselves

stimulate a change in peak metabolic rate. We have tested for this effect by

categorizing animals according to the number of runs to which they were

exposed prior to their initial peak run and analyzing for differences among

the categories in measured peaks using analysis of variance. Table 63

summarizes the data. At a robust level, there were no significant differences

in measured peak metabolic rate among these categories (P = 0.59 for deermice, 0.47 for chickadees). Thus, runs in helium-oxygen do not themselves seem to have altered measured peaks.

As earlier noted, target dates for retesting of the peak metabolic rate were 11-12 and 18 days after capture. However, logistical factors sometimes forced retesting to be done on other days. We have divided the retest runs on each species into two sets: early-retest and late-retest. Table 64 shows the time between capture and retest for the runs in these sets, as well as the time between capture and initial peak determination. The early- and late-retest sets for deermice included 33 and 24 runs, respectively. The mean time between capture and retesting for the two sets was 12.3 and 18.4 days. The mean time between capture and initial peak determination was 2.9-3.0 days in each case. Of the 40 individual deermice for which data were obtained, 13 contributed data just to the early-retest set, 20 contributed to both sets, and 4 contributed only to the late- retest set. The remaining 3 individuals contributed just to a special set discussed later. The early- and late-retest sets for chickadees included 28 and 23 runs, respectively. The mean time between capture and retesting for the two sets was 11.6 and 18.1 days. The mean time between capture and initial peak determination was 2.2 days in each case. Of the 32 individual chickadees for which data were obtained, 7 contributed data just to the early-retest set, 21 contributed to both sets, and 2 contributed only to the late-retest set. The remaining 2 individuals contributed just to the special set.

The data in the early- and late-retest sets were analyzed in accord with a blocked experimental design. Specifically, within each set, each individual animal was treated as a block. The dependent variable used in the analysis was total (whole-body) metabolic rate (see discussion). A two-way analysis of

variance was carried out on each set, with one factor being the type of run (initial peak or retest) and the other being block. The blocking removed effects on variance attributable to differences between individuals (e.g. interindividual differences in body weight); and, in this way, we were able to evaluate whether there was a difference between the initial peak metabolic rate and retest metabolic rate <u>within</u> individuals. Normality of the metabolic data was assessed using probit plots. The data for the late-retest set of deermice and both sets of chickadees were normal. However, those for the early-retest set of deermice had to be transformed to logarithms to achieve normality.

Table 65 summarizes the data. At a robust level, there were no significant differences between initial peak metabolic rates and retest metabolic rates in the late-retest set for deermice (P = 0.67) or in the early- or late-retest sets for chickadees (P = 0.20 and 0.62, respectively). The difference between initial peak metabolic rates and retest rates in the early-retest set for deermice was significant (P= 0.02). The retest rates averaged 3 percent higher than the initial peaks.

Table 66 presents six data sets that did not conform to the definitions of the early- or late-retest sets. The first individual in the table was retested at 29 days after capture. For the others, the initial peak was not determined until 8 days after capture. As a set, the data in Table 66 indicate that there was either no change or an increase in metabolic rate between the initial peak measurement and retesting, and thus the data are in conformity with the results from the early- and late- retest sets.

As earlier noted, body weight and body temperature can exert important influences on metabolic rates. Table 67 summarizes weights and final body temperatures in the early- and late-retest sets. The within-individual

differences between weight at initial peak determination and weight at retest in each retest set were examined using the Wilcoxon matched-pairs signed-ranks test (normal statistics could not be used because the data and their logarithmic transforms were not normal). Weight increased significantly between initial peak determination and retesting in both early-retest sets (P = 0.001 for deermice, 0.047 for chickadees). However, the average increases in weight were small: 3.6 and 1.5% for the deermice and chickadees, respectively (calculations carried out on unrounded values). Weight did not change significantly between initial peak determination and retest in either late-retest set (P = 0.07 for deermice, 0.29 for chickadees). The within-individual differences between final body temperature at initial peak determination and temperature at retest in each retest set were also examined using the Wilcoxon test (except that analysis of variance was used for the deermouse early-retest set because those data alone met the assumption of normality). In the early-retest set for deermice, final body temperature was significantly higher at retest that at initial peak determination (P = 0.002). However, the body temperature at retest was not significantly different from that at initial peak determination in any of the other retest sets (P = 0.27 - 0.85).

Final analysis of captivity studies: Discussion. Initial peak metabolic rates measured 1-5 days after capture did not differ as a function of the number of days after capture in either species. Animals retested 9-14 days after capture (early- retest sets) exhibited peak metabolic rates that were an average of 3% higher than initial peak rates in the case of deermice but that did not differ from initial peak rates in the case of chickadees. The metabolic rates at retest of animals retested 16 - 22 days after capture (late-retest sets) did not differ from initial peak metabolic rates in either

species. Overall, these results indicate that the peak metabolic rates of deermice and chickadees captured in winter do not change during three weeks of captivity in outdoor cages. In turn, the results indicate that time in captivity is not a confounding factor in either our routine determinations of peak metabolic rates (which typically take a week or less from time of capture) or in our comparisons of peak metabolic rates between animals from test and control plots. The peak metabolic rates we measure are representative of the peak rates of free-living animals insofar as we can assess the matter experimentally.

We analyzed the early- and late-retest sets by comparing the total (whole-body) metabolic rates of individuals at initial peak determination and retest. We used total rates rather than weight-specific rates in these analyses because changes in weight-specific rates between initial peak determination and retest would likely confound two entirely different processes: changes in the metabolic capacity of preexisting tissues and changes in body weight. We have reasoned that measured weight changes within individuals over the 3 weeks of our study were likely attributable mostly to changes in the body content of substances that have relatively low (or zero) metabolic rates per unit weight: white fat, water, and gut contents. Because changes in body weight attributable to such relatively inert substances would not have proportionate effects on peak metabolic output, an examination of changes in weight-specific metabolic rates would have the confounding effects already specified. An analysis of changes in the total metabolic rates of individuals provides the more straightforward insight into changes in the metabolic capacity of preexisting tissues. Provided the preexisting tissues retain their original metabolic capacities, the total peak metabolic rate should remain nearly

constant despite changes in body weight if the changes in weight are attributable to changes in body content of metabolically inert substances.

When animals change in weight during the study period, it is impossible to escape entirely the problem that, in metabolic measures, effects of weight on metabolism and effects of tissue metabolic competence are confounded. Our first line of defense against an excessive confounding of these factors in our results was to exclude from study animals that increased in weight by more than 15%. Our second line of defense has been to examine weight changes statistically so that significant changes could be identified and taken into account.

Body weight increased significantly over the study period in the chickadee early-retest set. Although there was no significant change in total peak metabolic rate in this set, the trend in total metabolic rate over the study period was downward; and it might be argued that, if the effect of the increase in weight on total metabolic rate could be eliminated, the downward trend in total metabolic rate (then magnified) would prove significant. However, the increase in weight was only 1.5% and thus could obscure only a very modest drop in the peak metabolic rate of the preexisting tissues.

The other group of animals that exhibited a significant change in weight was the deermouse early-retest set. This was also the one set to display a significant change in total peak metabolic rate over the study period, and it was the only set for which final body temperatures at retesting differed significantly from temperatures at initial peak determination. Specifically, between initial peak determination and retesting, total metabolic rate increased 3.3%, body weight increased 3.6%, and temperature increased 1.2°C (sufficient to support a 9% increase in metabolic rate according to the van't Hoff equation, assuming a Q_{10} of 2). All this could be taken to indicate that

the increase in metabolic rate resulted from increases in weight and maintained body temperature rather than from changes in the intrinsic metabolic capacity of preexisting tissues, and in fact an argument could be made for a modest decrease in intrinsic metabolic capacity. A firm conclusion cannot be reached. It is clear, however, that any changes that occurred in the metabolic competence of preexisting tissues -- positive or negative -- were small.

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CONCLUSION

In conclusion, we are now in a good position to compare data from pre-antenna years with those during antenna testing at greatly reduced output and with those to come and full antenna operation. We have data from plots including the antenna line and plots away from the antenna (controls). Our findings to date show, for the most part, that our plots do not differ in the many variables we are measuring. In a few cases, we have found differences that could be due to the antenna system. Specifically homing frequency in tree swallows and deermice and growth of tarsus in tree swallow nestlings. We plan careful examination of future data to further assess these findings. In other cases, we have found differences with no discernible relation to the antenna. We will continue to evaluate these findings as well.

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APPENDIX A - TABLES AND FIGURES

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Table 1. Test-control plot pairings for the various work elements for small mammals and nesting birds. Plot code designations are those used by IITRI.

STUDY ELEMENT	TEST PLOT	CONTROL PLOT
Deermouse Growth & Maturation	PIRLOT ROAD (1T1)	MICHIGAMME NORTH (1C1)
Small Mammal Homing	PIRLOT ROAD (1T1)	MICHIGAMME SOUTH MICHIGAMME NORTH (1C3, 1C1)
Deermouse Winter Physiology	PIRLOT ROAD (1T1)	MICHIGAMME SOUTH (1C1)
Tree Swallow Growth & Maturation	PIRLOT ROAD (1T1)	TACHYCINETA MEADOW (1C6)
Tree Swallow Homing (Home Plots)	CLEVELAND HOMESTEAD (1T2)	PANOLA PLAINS (1C4)
	NORTH TURNER ROAD (1T4)	PANOLA PLAINS (1C4)
(Displacement Plots)	CLEVELAND HOMESTEAD DISPLACEMENT (1D1)	-
	NORTH TURNER DISPLACEMENT (1D2)	-
	-	PANOLA PLAINS DISPLACEMENT (1D3)
Tree Swallow Embryology	CLEVELAND HOMESTEAD (1T2)	TACHYCINETA MEADOW (1C6)
	FORD RIVER NORTH (1T5)	PANOLA PLAINS (1C4)
	FORD RIVER SOUTH (1T6)	
Black-capped Chickadee Winter Physiology	PIRLOT ROAD (1T1)	MICHIGAMME NORTH (1C1)

Note: Cleveland Homestead, Ford River North and Ford River South plots are small. Therefore they have been designated solely as tree swallow embryology study sites.

Table 2. Mean values for 60 Hz transverse electric fields (V/m) on test and control plots for years 1983 to 1988. The values in parentheses are the sample n. Values listed by IITRI as <0.001 are treated as equal to 0.001. Plot 1D3 is the release site for tree swallows used in homing studies on control plots, and plots 1D1 and 1D2 are release sites used for test plots.

PLOT	1983	1984	1985	1986	1987	1988
CONTROLS						
1C1	0.001 (1)	0.001 (1)	0.001 (2)	0.001 (2)	0.001 (2)	0.001 (2)
1C3	0.001 (2)	0.001 (2)	0.001 (1)	0.001 (2)	0.001 (2)	0.001 (2)
1C4	-	0.001 (3)	0.001 (4)	0.001 (3)	0.001 (3)	0.001 (3)
1C6	-	0.001 (1)	0.001 (3)	0.001 (3)	0.001 (3)	0.001 (3)
Average	0.001 (3)	0.001 (7)	0.001 (10)	0.001 (10)	0.001 (10)	0.001 (10)
1D3	-	-	-	0.001 (1)	0.001 (1)	0.001 (1)
TESTS						
1T1	0.001 (1)	0.001 (4)	0.001 (6)	0.001 (14)	0.007 (14)	0.028 (18)
1T2	0.001 (1)	0.001 (1)	0.001 (1)	0.001 (4)	0.042 (6)	0.013 (5)
1T4	-	0.001 (1)	0.001 (3)	0.001 (5)	0.020 (10)	0.014 (10)
1T5	0.001 (1)	0.001 (2)	0.001 (2)	0.001 (6)	0.026 (9)	0.037 (9)
1T6	0.001 (1)	0.001 (2)	0.001 (1)	0.001 (1)	0.028 (7)	0.078 (7)
Average	0.001 (4)	0.001 (10)	0.001 (13)	0.001 (30)	0.021 (46)	0.034 (49)
1D1 & 1D2 (Average)	-	-	-	1.251 (2)	0.001 (2)	4.601 (2)

Table 3. Mean values for 60 Hz longitudinal electric fields (mV/m) on
test and control plots for years 1983 to 1988. The values in
parentheses are the sample n. Plot 1D3 is the release site
for tree swallows used in homing studies on control plots,
and plots 1D1 and 1D2 are release sites used for test plots.

PLOT	1983	1984	1985	1986	1987	1988
CONTROLS						
1C1	0.041 (1)	0.146 (1)	0.092 (2)	0.100 (2)	0.114 (2)	0.338 (2)
1C3	0.115 (2)	0.226 (2)	0.133 (1)	0.080 (2)	0.148 (2)	0.117 (2)
1C4	-	0.034 (3)	0.044 (4)	0.065 (3)	0.052 (2)	0.048 (3)
1C6	-	0.072 (1)	0.085 (3)	0.068 (3)	0.089 (3)	0.041 (3)
Average	0.091 (3)	0.110 (7)	0.075 (10)	0.076 (10)	0.099 (9)	0.136 (10)
1D3	-	-	-	0.052 (1)	0.156 (1)	0.053 (1)
TESTS						
1T1	0.090 (1)	0.143 (4)	0.116 (6)	0.070 (14)	0.070 (14)	0.252 (18)
1T2	0.170 (1)	0.220 (1)	0.197 (1)	0.074 (4)	0.059 (5)	0.075 (5)
1T4	-	0.181 (1)	0.167 (3)	0.086 (5)	0.076 (10)	0.110 (10)
1T5	0.230 (1)	0.295 (2)	0.235 (2)	0.079 (6)	0.078 (9)	0.159 (9)
1T6	0.071 (1)	0.765 (1)	0.870 (1)	0.230 (1)	0.297 (7)	1.324 (7)
Average	0.140 (4)	0.259 (9)	0.210 (13)	0.080 (30)	0.108 (45)	0.384 (49)
1D1 & 1D2 (Average)	-	-	-	5.035 (2)	1.280 (2)	0.715 (2)

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Table 4. Mean values for 60 Hz magnetic fields (mG) on test and
          control plots for years 1983 to 1988. The values in
          parentheses are the sample n. Values listed by IITRI as
          <0.001 are treated as equal to 0.001. Plot 1D3 is the
          release site for tree swallows used in homing studies on
          control plots, and plots 1D1 and 1D2 are release sites used
          for test plots.

PLOT	1983	1984	1985	1986	1987	1988
CONTROLS						
1C1	0.001 (1)	0.001 (1)	0.001 (2)	0.001 (2)	0.001 (2)	0.001 (2)
1C3	0.001 (2)	0.003 (2)	0.002 (1)	0.001 (2)	0.001 (2)	0.001 (2)
1C4	-	0.001 (3)	0.002 (4)	0.001 (3)	0.002 (2)	0.001 (3)
1C6	-	0.003 (1)	0.003 (3)	0.003 (3)	0.003 (3)	0.002 (3)
Average	0.001 (3)	0.002 (7)	0.002 (10)	0.002 (10)	0.002 (9)	0.001 (10)
1D3	-	-	-	0.003 (1)	0.002 (1)	0.002 (1)
TESTS						
1T1	0.002 (1)	0.003 (4)	0.003 (6)	0.009 (14)	0.010 (14)	0.052 (18)
1T2	0.001 (1)	0.001 (1)	0.001 (1)	0.025 (4)	0.018 (5)	0.010 (5)
1T4	-	0.001 (1)	0.001 (3)	0.012 (5)	0.021 (10)	0.018 (10)
1T5	0.001 (1)	0.002 (2)	0.001 (2)	0.018 (6)	0.026 (9)	0.047 (9)
1T6	0.002 (1)	0.001 (1)	0.001 (1)	0.020 (1)	0.033 (7)	0.094 (7)
Average	0.002 (4)	0.002 (9)	0.002 (13)	0.014 (30)	0.020 (45)	0.044 (49)
1D1 & 1D2 (Average)	-	-	-	0.057 (2)	0.080 (2)	0.023 (2)

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Table 5. Comparison of values for 60 Hz fields on test and control plots and the corresponding release plots used for tree swallow homing in 1986 through 1988. Fields are coded as T - transverse fields (V/m), L - longitudinal fields (mV/m) and M - magnetic fields (mG).

Plot	EM Field:	Ratio of larger/smaller T	L	M
Control and Release plot:				
Panola Plains (1C4)				
and release plot 1D3: 1986		1.0	1.3	3.0
1987		1.0	3.0	1.0
1988		1.0	1.1	2.0
Test and Release plot:				
Cleveland (1T2) and				
release plot 1D1: 1986		2500.0	184.6	4.4
1987		47.0	40.7	8.6
1988		707.7	15.3	4.0
North Turner (1T4) and				
release plot 1D2: 1986		1.0	5.9	3.0
1987		20.0	2.1	3.5
1988		14.0	2.5	3.6

Table 6. Mean values for 76 Hz transverse electric fields (V/m) on test and control plots for years 1986 (4 or 6 amperes), 1987 (15 amperes), and 1988 (75 amperes). The value in parentheses is the sample N. NS refers to the north-south antenna segment; EW to the averaged east-west segments in 1986 and a single measurement for simultaneous operation of both east-west segments in 1987. All measures on test plots were made on the NS segment, except for displacement plots 1D1 and 1D2, which are located north of the northernmost EW segment. All values reported by IITRI as <0.001 were set to 0.001.

| | TRANSVERSE FIELDS (V/m) | | | | | |
| | 1986 (4 or 6 amp) Antenna | | 1987 (15 amps) Antenna | | 1988 (75 amps) Antenna | |
PLOT	NS	EW	NS	EW	NS	EW
CONTROLS						
1C1	0.001	0.001 (2)	0.001	0.001 (2)	0.001	0.001 (2)
1C3	0.001	0.001 (2)	0.001	0.001 (2)	0.001	0.001 (2)
1C4	0.001	0.001 (4)	0.001	0.001 (3)	0.001	0.001 (3)
1C6	0.001	0.001 (3)	0.001	0.001 (3)	0.001	0.001 (3)
Average	0.001	0.001 (11)	0.001	0.001 (10)	0.001	0.001 (10)
1D1	0.001	0.001 (1)	0.001	0.001 (1)	0.001	0.001 (1)
TESTS						
1T1	0.078	0.001 (14)	0.264	0.001 (14)	0.897	0.004 (18)
1T2	0.085	0.001 (4)	0.301	0.004 (5)	1.710	0.025 (5)
1T4	0.140	0.001 (5)	0.426	0.001 (10)	1.936	0.011 (10)
1T5	0.237	0.001 (6)	0.790	0.002 (9)	3.614	0.013 (9)
1T6	0.182	0.001 (1)	0.544	0.002 (7)	9.912	0.011 (7)
Average	0.125	0.001 (30)	0.453	0.002 (45)	2.014	0.013 (49)
1D1 & 1D2 (Average)	0.001	0.002 (2)	0.002	0.010 (2)	0.001	0.001 (2)

Table 7. Mean values for 76 Hz longitudinal electric fields on test
and control plots for years 1986 (4 or 6 amperes), 1987 (15
amperes), and 1988 (75 amperes). The value in parentheses is
the sample N. NS refers to the north-south antenna segment;
EW to the averaged east-west segments in 1986 and a single
measurement for simultaneous operation of both east-west
segments in 1987. All measures on test plots were made on
the NS segment, except for displacement plots 1D1 and 1D2,
which are located north of the northernmost EW segment.
All values reported by IITRI as <0.001 were set to 0.001.

| | LONGITUDINAL FIELDS (mV/m) | | | | | |
| | 1986 (4 or 6 amps) Antenna | | 1987 (15 amps) Antenna | | 1988 (75 amps) Antenna | |
PLOT	NS	EW	NS	EW	NS	EW
CONTROLS						
1C1	0.021	0.006 (1)	0.085	0.031 (2)	0.430	0.162 (2)
1C3	0.022	0.008 (1)	0.068	0.029 (2)	0.335	0.138 (2)
1C4	0.001	0.001 (1)	0.003	0.003 (3)	0.013	0.011 (3)
1C6	0.001	0.001 (1)	0.005	0.003 (3)	0.020	0.014 (3)
Average	0.011	0.004 (4)	0.033	0.014 (10)	0.200	0.081 (10)
1D3	0.008	0.004 (1)	0.053	0.019 (1)	0.210	0.065 (1)
TESTS						
1T1	1.089	0.030 (1)	4.244	0.070 (14)	19.900	0.424 (18)
1T2	1.705	0.128 (14)	7.500	0.728 (5)	34.600	3.520 (5)
1T4	2.162	0.082 (4)	7.390	0.303 (10)	36.300	1.387 (10)
1T5	1.958	0.072 (5)	6.600	0.229 (9)	28.444	0.977 (9)
1T6	5.400	0.122 (6)	18.457	0.184 (7)	83.857	0.920 (7)
Average	1.668	0.063 (30)	7.987	0.244 (45)	40.620	1.446 (49)
1D1 & 1D2 (Average)	0.068	0.225 (2)	0.320	1.015 (2)	1.365	4.100 (2)

Table 8.   Mean values for 76 Hz magnetic fields (mG) on test and
control plots for years 1986 (4 or 6 amperes), 1987 (15
amperes), and 1988 (75 amperes).   The value in parentheses
is the sample N.   NS refers to the north-south antenna
segment;   EW refers to the averaged east-west segments in
1986 and a single measurement for simultaneous operation of
both east-west segments thereafter.   All measures on test
plots were made on the NS segment, except for displacement
plots 1D1 and 1D2, which are located north of the
northernmost EW segment.   All values reported by IITRI as
<0.001 were set to 0.001.

| | MAGNETIC FIELDS (mG) | | | | | |
| | 1986 (4 or 6 amps) Antenna | | 1987 (15 amps) Antenna | | 1988 (75 amps) Antenna | |
PLOT	NS	EW	NS	EW	NS	EW
CONTROLS						
1C1	0.001	0.001 (1)	0.001	0.001 (2)	0.003	0.001 (2)
1C3	0.001	0.001 (1)	0.001	0.001 (2)	0.003	0.001 (2)
1C4	0.001	0.001 (1)	0.001	0.001 (3)	0.001	0.001 (3)
1C6	0.001	0.001 (1)	0.001	0.001 (3)	0.001	0.001 (3)
Average	0.001	0.001 (4)	0.001	0.001 (10)	0.002	0.001 (10)
1D3	0.001	0.001 (1)	0.001	0.001 (1)	0.002	0.002 (1)
TESTS						
1T1	0.143	0.003 (14)	0.530	0.001 (14)	2.251	0.015 (18)
1T2	0.301	0.005 (4)	1.164	0.003 (5)	5.538	0.029 (5)
1T4	0.173	0.003 (4)	1.050	0.002 (10)	5.410	0.019 (10)
1T5	0.337	0.007 (6)	1.409	0.002 (9)	6.600	0.025 (9)
1T6	0.400	0.007 (1)	1.043	0.005 (7)	4.889	0.016 (7)
Average	0.225	0.004 (29)	0.972	0.003 (45)	4.938	0.021 (49)
1D1 & 1D2 (Average)	0.001	0.002 (2)	0.002	0.010 (2)	0.007	0.048 (2)

Table 9. Comparison of values for 76 Hz fields on test and control plots and the corresponding release plots used for tree swallow homing in 1986, 1987 and 1988. Fields are coded as T - transverse fields (V/m), L - longitudinal fields (mV/m)· and M - magnetic fields (mG). Values are averaged for NS and EW antenna segment operation.

Plot	Field:	Ratio Larger/smaller		
		T	L	M
Control and Release plot:				
Panola Plains (1C4)				
and release plot 1D3:	1986	1.0	6.0	1.0
	1987	1.0	12.0	1.0
	1988	1.0	11.5	2.0
Test and Release plots:				
Cleveland (1T2)				
and release plots :	1986	21.5	6.2	76.5
(1D1 & 1D2)	1987	25.4	6.2	97.3
	1988	867.5	7.0	·101.8
North Turner (1T4)				
and release plots :	1986	35.3	7.6	44.0
(1D1 & 1D2)	1987	35.6	11.5	87.7
	1988	973.5	6.9	98.7

Table 10. Tree swallow plots, number of boxes, and percent with egg laying activity on test and control sites for 1985, 1986, 1987, 1988, and 1989. Egg laying activity is defined as at least two eggs laid before abandonment or continuation of nesting.

PLOT NAME	NUMBER OF BOXES	% ACTIVITY				
		1985	1986	1987	1988	1989
CLEVELAND HOMESTEAD TEST	38	58	62	66	74	68
FORD NORTH TEST	17	30	47	41	47	41
FORD SOUTH TEST	20	25	55	70	55	70
NORTH TURNER TEST	47	23	60	70	68	60
PIRLOT ROAD TEST	36	75	72	78	75	83
PANOLA PLAINS CONTROL	124	43	77	87	85	90
TACHYCINETA MEADOWS CONTROL	75	43	69	79	85	92
TOTALS TEST	158	44	61	68	68	66
CONTROL	199	43	73	83	85	90

Note: 24 nestboxes were added in 1988 and in 1989

Table 11. Tree swallow fecundity data for years 1989, 1988, 1987, 1986 and 1985. Data are from the Pirlot 'Road test plot and Tachycineta Meadows control plot and excludes any renests which may have occurred.

Variable	Year	TEST n	$\bar{X}$	SD	CONTROL n	$\bar{X}$	SD
Clutch Size"	1989	27	5.1	0.91	69	5.4	0.84
	1988	26	5.4	0.81	61	5.3	0.85
	1987	24	5.0	0.75	55	5.2	0.81
	1986	23	5.3	0.88	48	4.9	1.01
	1985	21	5.4	0.87	19	4.8	0.86
Hatch Rate""	1989	20	4.3	1.29	51	4.2	1.37
	1988	18	5.0	0.84	43	4.8	1.23
	1987	15	4.2	1.32	40	4.2	1.25
	1986	14	5.1	1.54	30	4.4	1.35
	1985	11	4.4	1.12	10	4.3	1.06
Fledge Rate"""	1989	20	0.8	1.45	50	0.9	1.73
	1988	16	4.3	1.49	37	3.3	2.14
	1987	14	3.1	1.99	39	3.1	1.85
	1986	14	1.3	2.27	27	1.2	2.00
	1985	10	3.6	0.84	7	2.6	1.90

Test of Frequency of Clutch Size[a]		G	df	P
	1989	1.5	2	>0.1
	1988	0.3	2	>0.3
	1987	2.6	2	>0.1
	1986	3.3	4	>0.3
	1985	5.4	3	>0.1

"   Clutch size is the maximum number of eggs laid in a nest.
""   Hatch rate is the number of eggs which hatch of those available to hatch -- not always the maximum number of eggs in the nest due to occasional predation.
"""   Fledge rate is the number of young that fledge from the eggs which hatch, and only include those nests which were followed to completion.
[a]   Categories of clutch size with fewer than 5 nests were not included.

Table 12. Likelihood to hatch and fledge for tree swallows for 1985
through 1989. Data are from the Pirlot Road test plot and
Tachycineta Meadows control plot. Overall comparisons were
made using the G test of independence (df=7).

** HATCHING SUCCESS **

Year	Plot	Hatch	Not Hatch	% Hatching
1989	Test	85	17	83.3
	Control	216	43	83.4
1988	Test	90	8	91.8
	Control	206	21	90.7
1987	Test	63	11	85.1
	Control	166	32	83.8
1986	Test	71	5	93.4
	Control	132	25	84.1
1985	Test	48	8	85.7
	Control	43	5	89.6

Overall G = 12.70   P > 0.10

** FLEDGING SUCCESS **

Year	Plot	Fledge	Not Fledge	% Fledging
1989	Test	15	70	17.6
	Control	47	165	22.2
1988	Test	69	12	85.2
	Control	123	55	69.1
1987	Test	44	17	72.1
	Control	122	39	75.8
1986	Test	18	53	25.4
	Control	32	86	27.1
1985	Test	36	7	83.7
	Control	18	13	58.1

Overall G = 316.55   P < 0.0001

Table 13. ANOVA for clutch size of tree swallows. Tested are the
effects of PLOT (test and control), YEAR (1985 through 1989)
and the interaction of PLOT and YEAR.

SOURCE	DF	TYPE III SS	MS	F	P > F
PLOT	1	1.265	1.265	1.72	0.191
YEAR	4	3.416	0.854	1.16	0.328
PLOT*YEAR	4	6.072	1.518	2.06	0.085
ERROR	364	267.822	0.736		

Table 14. ANOVA for hatch success of tree swallows. Tested are the
effects of PLOT (test and control), YEAR (1985 through 1989)
and the interaction of PLOT and YEAR.

SOURCE	DF	TYPE III SS	MS	F	P > F
PLOT	1	1.365	1.365	0.85	0.358
YEAR	4	21.219	5.305	3.29	0.012
PLOT*YEAR	4	3.629	0.907	0.56	0.690
ERROR	247	397.853	1.611		

Table 15. ANOVA for fledging success of tree swallows. Tested are the
effects of PLOT (test and control), YEAR (1985 through 1989)
and the interaction of PLOT and YEAR.

SOURCE	DF	TYPE III SS	MS	F	P > F
PLOT	1	5.671	5.671	1.68	0.197
YEAR	4	304.326	76.082	22.51	0.0001
PLOT*YEAR	4	12.747	3.187	0.94	0.440
ERROR	227	767.216	3.652		

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Table 16. Age in days at landmark events of eye opening and primary
feather eruption in 1986 through 1989. Data are from the
Pirlot Road test plot and Tachycineta Meadows control plot.
Sample sizes are numbers of individual young. Day of
hatching is defined as day zero.

Year	Plot	Eye Opening			Primary Eruption		
		n	$\bar{X}$	SD	n	$\bar{X}$	SD
1989	Test	25	8.6	1.16	25	9.1	1.69
	Control	32	7.8	0.93	32	9.6	1.06
1988	Test	76	7.3	1.36	76	8.2	1.21
	Control	74	6.7	1.38	74	8.8	1.25
1987	Test	44	7.4	1.84	44	8.5	1.13
	Control	66	6.7	1.48	66	8.5	1.40
1986	Test	18	5.1	1.02	18	8.8	1.11
	Control	42	6.0	0.73	42	9.1	1.52

Table 17. Nested ANOVA for age of eye opening in tree swallows. Tested are the effects of PLOT (test or control), and nests (NEST) within a plot (PLOT) for 1986 through 1989.

YEAR	SOURCE	DF	TYPE III SS	MS	F	P > F
1989	PLOT	1	5.277	5.227	2.10	0.166
	NEST(PLOT)	16	40.113	2.507	5.05	0.0001
	ERROR	39	19.379	0.497		
1988	PLOT	1	10.654	10.654	1.54	0.225
	NEST(PLOT)	28	194.146	6.934	9.90	0.0001
	ERROR	120	84.050	0.700		
1987	PLOT	1	9.742	9.742	1.13	0.298
	NEST(PLOT)	24	206.892	8.621	9.12	0.0001
	ERROR	84	79.433	0.946		
1986	PLOT	1	3.806	3.806	2.76	0.123
	NEST(PLOT)	12	16.566	1.380	2.75	0.007
	ERROR	46	23.117	0.502		

Table 18. Nested ANOVA for primary feather eruption in tree swallows. Tested are the effects of PLOT (test or control), and nests (NEST) within a plot (PLOT) for 1986 through 1989.

YEAR	SOURCE	DF	TYPE III SS	MS	F	P > F
1989	PLOT	1	1.523	1.523	0.33	0.575
	NEST(PLOT)	16	74.545	4.659	6.40	0.0001
	ERROR	39	28.413	0.729		
1988	PLOT	1	15.319	15.319	2.66	0.114
	NEST(PLOT)	28	161.410	5.765	11.10	0.0001
	ERROR	120	62.317	0.519		
1987	PLOT	1	0.038	0.038	0.01	0.938
	NEST(PLOT)	24	147.644	6.152	15.27	0.0001
	ERROR	84	33.833	0.403		
1986	PLOT	1	0.460	0.460	0.07	0.797
	NEST(PLOT)	12	80.071	6.673	8.48	0.0001
	ERROR	46	36.183	0.787		

Table 19. Detectable differences and power for tree swallow fecundity
variables: clutch size, hatch success and fledging success
for years 1985 - 1989. N = number of nests per treatment for
test or control. Differences presented in units of variable
with % of grand mean following.

Variable	N	Actual Detectable Difference (%)	Actual Power	Detectable Difference at 70% Power (%)
Clutch size eggs(%)	182	.076(1.5)	<.30	.22(4.2)
Hatch success eggs(%)	123	.063(1.4)	<.30	.40(9.0)
Fledging success young(%)	113	.201(8.9)	<.30	.61(27.0)

Table 20. Detectable differences and power for tree swallow landmark
events; eye opening and feather eruption, for years 1986
through 1989. N = number of nests per treatment for test or
control. Differences presented in days with % of grand mean
following.

Variable	Year	N	Actual Detectable Difference(%)	Actual Power	Detectable Difference at 70% Power (%)
Eye opening days(%)	1989	8	.83(10.1)	<.30	2.08(25.5)
	1988	14	.73(10.4)	<.30	2.55(36.4)
	1987	12	.43(6.2)	<.30	3.15(45.1)
	1986	6	.90(15.7)	<.30	1.84(32.1)
Feather eruption days(%)	1989	8	.885(9.4)	<.30	2.85(30.4)
	1988	14	1.168(13.7)	<.30	2.35(27.6)
	1987	12	1.009(11.9)	<.30	2.65(31.2)
	1986	6	1.439(16.0)	<.30	4.05(45.0)

Table 21. Exposure data and frequency of mortality for EGGS for all
years calculated using the Mayfield method (Mayfield 1961,
1975). Data are pooled from all test and control sites.
Comparisons between test and control were calculated using
G-tests (Sokal and Rohlf 1981).

TEST

Year	Egg Exposure Days	Egg Mortalities
1989	8112	179
1988	8212	135
1987	6457	216
1986	5821	139
1985	2843	37

CONTROL

Year	Egg Exposure Days	Egg Mortalities
1989	17992	336
1988	12284	144
1987	10195	210
1986	7265	248
1985	2695	34

Table 22. Exposure data and frequency of mortality for NESTLINGS for
all years calculated using the Mayfield method (Mayfield
1961, 1975). Data are pooled from all test and control
sites. Comparisons between test and control were calculated
using G-tests (Sokal and Rohlf 1981).

TEST

Year	Nestling Exposure Days	Nestling Mortalities
1989	3614	226
1988	4755	115
1987	3049	41
1986	2377	135
1985	1941	14

CONTROL

Year	Nestling Exposure Days	Nestling Mortalities
1989	6974	495
1988	10159	116
1987	7899	87
1986	3100	104
1985	1463	22

Table 23. Exposure data and frequency of mortality for OVERALL NESTS for all years calculated using the Mayfield method (Mayfield 1961, 1975). Data are pooled from all test and control sites. Comparisons between test and control were calculated using G-tests (Sokal and Rohlf 1981).

TEST

Year	Nest Exposure Days	Nest Mortalities
1989	2839	73
1988	3280	31
1987	2467	46
1986	1780	45
1985	1093	11

CONTROL

Year	Nest Exposure Days	Nest Mortalities
1989	6215	138
1988	4965	28
1987	4510	47
1986	2385	64
1985	940	23

Table 24. Exposure data and frequency of mortality for INCUBATION PHASE NEST MORTALITY for all years calculated using the Mayfield method (Mayfield 1961, 1975). Data are pooled from all test and control sites. Comparisons between test and control were calculated using G-tests (Sokal and Rohlf 1981).

TEST

Year	Nest Exposure Days	Nest Mortalities
1989	1821	27
1988	1735	18
1987	1598	38
1986	1242	21
1985	583	7

CONTROL

Year	Nest Exposure Days	Nest Mortalities
1989	3966	41
1988	2678	18
1987	2563	31
1986	1628	39
1985	579	17

Table 25. Exposure data and frequency of mortality for NESTLING PHASE NEST MORTALITY for all years calculated using the Mayfield method (Mayfield 1961, 1975). Data are pooled from all test and control sites. Comparisons between test and control were calculated using G-tests (Sokal and Rohlf 1981).

TEST

Year	Nest Exposure Days	Nest Mortalities
1989	1008	46
1988	1164	13
1987	867	8
1986	539	24
1985	510	4

CONTROL

Year	Nest Exposure Days	Nest Mortalities
1989	2247	97
1988	2352	10
1987	1947	16
1986	757	25
1985	361	6

Table 26. Nested ANOVA for weight growth constant for nestling tree
swallows. Tested are the effects of PLOT (test or control),
and nests (NEST) within a plot (PLOT) for 1985 through 1989.

YEAR	SOURCE	DF	TYPE III SS	MS	F	P > F
1989	PLOT	1	0.000007	0.000007	<0.00	0.984
	NEST(PLOT)	14	0.2330	0.01664	5.06	0.0001
	ERROR	38	0.1251	0.00329		
1988	PLOT	1	0.0504	0.0504	2.87	0.101
	NEST(PLOT)	28	0.4916	0.01756	3.03	0.0001
	ERROR	112	0.6486	0.00579		
1987	PLOT	1	0.00004	0.00004	0.00	0.968
	NEST(PLOT)	24	0.565	0.024	4.23	0.0001
	ERROR	80	0.445	0.006		
1986	PLOT	1	0.0009	0.0009	0.13	0.724
	NEST(PLOT)	12	0.090	0.007	2.43	0.015
	ERROR	46	0.141	0.003		
1985	PLOT	1	0.000009	0.000009	0.00	0.987
	NEST(PLOT)	23	0.722	0.031	8.37	0.0001
	ERROR	80	0.300	0.004		

Table 27. Nested ANOVA for the inflection point of weight increase in
tree swallows. Tested are the effects of PLOT (test vs
control), and nests (NEST) within a plot for 1985 through
1989.

YEAR	SOURCE	DF	TYPE III SS	MS	F	P > F
1989	PLOT	1	1.089	1.089	0.51	0.484
	NEST(PLOT)	14	29.612	2.115	5.49	0.0001
	ERROR	38	14.654	0.386		
1988	PLOT	1	1.13786	1.13786	0.55	0.466
	NEST(PLOT)	28	58.39058	2.085378	4.37	0.0001
	ERROR	112	53.3859	0.47666		
1987	PLOT	1	1.563	1.563	0.67	0.423
	NEST(PLOT)	24	56.394	2.350	5.51	0.0001
	ERROR	80	34.094	0.426		
1986	PLOT	1	0.004	0.004	0.00	0.955
	NEST(PLOT)	12	15.241	1.270	3.80	0.0005
	ERROR	46	15.390	0.335		
1985	PLOT	1	3.190	3.190	1.62	0.216
	NEST(PLOT)	23	45.226	1.966	7.12	0.0001
	ERROR	80	22.080	0.276		

Table 28. Nested ANOVA for tarsus growth constant in tree swallows.
Tested are the effects of PLOT (test vs control) and nests
(NEST) within a plot for 1985 through 1989.

YEAR	SOURCE	DF	TYPE III SS	MS	F	P > F
1989	PLOT	1	0.029	0.029	8.47	0.011 *
	NEST(PLOT)	14	0.047	0.003	3.01	0.004
	ERROR	38	0.043	0.001		
1988	PLOT	1	0.005	0.005	0.62	0.439
	NEST(PLOT)	26	0.191	0.007	1.68	0.35
	ERROR	102	0.444	0.004		
1987	PLOT	1	0.001	0.001	0.10	0.753
	NEST(PLOT)	24	0.254	0.011	2.94	0.0002
	ERROR	74	0.266	0.004		
1986	PLOT	1	0.0315	0.0315	2.96	0.111
	NEST(PLOT)	12	0.128	0.0106	4.48	0.0001
	ERROR	44	0.105	0.002		
1985	PLOT	1	0.014	0.014	0.43	0.518
	NEST(PLOT)	23	0.722	0.031	6.50	0.0001
	ERROR	80	0.386	0.005		

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* Significant F value

Table 29. Nested ANOVA for the inflection point of tarsus growth in tree swallows. Tested are the effects of PLOT (test vs control), and nests (NEST) within a plot for 1985 through 1989.

YEAR	SOURCE	DF	TYPE III SS	MS	F	P > F
1989	PLOT	1	1.132	1.132	0.45	0.514
	NEST(PLOT)	14	35.372	2.567	14.76	0.0001
	ERROR	38	6.505	0.171		
1988	PLOT	1	0.043	0.043	0.04	0.844
	NEST(PLOT)	26	28.703	1.104	1.48	0.086
	ERROR	102	76.045	0.7455		
1987	PLOT	1	0.645	0.645	0.10	0.751
	NEST(PLOT)	24	149.639	6.235	5.12	0.0001
	ERROR	74	90.149	1.218		
1986	PLOT	1	1.917	1.917	0.65	0.437
	NEST(PLOT)	12	35.615	2.968	3.80	0.0006
	ERROR	44	34.399	0.782		
1985	PLOT	1	0.538	0.538	0.21	0.648
	NEST(PLOT)	23	57.860	2.516	4.22	0.0001
	ERROR	80	47.662	0.596		

Table 30. Nested ANOVA for ulna growth in tree swallows. Tested are
the effects of PLOT (test vs control), and nests (NEST)
within a plot for 1985 through 1989.

YEAR	SOURCE	DF	TYPE III SS	MS	F	P > F
1989	PLOT	1	0.003	0.003	0.69	0.420
	NEST(PLOT)	14	0.058	0.004	2.86	0.005
	ERROR	38	0.055	0.001		
1988	PLOT	1	0.007	0.007	0.83	0.369
	NEST(PLOT)	28	0.223	0.008	4.15	0.0001
	ERROR	117	0.225	0.002		
1987	PLOT	1	0.0006	0.0006	0.17	0.683
	NEST(PLOT)	24	0.081	0.003	1.29	0.197
	ERROR	80	0.208	0.003		
1986	PLOT	1	0.00007	0.00007	0.02	0.891
	NEST(PLOT)	12	0.0443	0.004	1.21	.306
	ERROR	45	0.137	0.003		
1985	PLOT	1	0.020	0.020	0.56	0.462
	NEST(PLOT)	23	0.817	0.036	10.76	0.0001
	ERROR	80	0.264	0.003		

Table 31. Nested ANOVA for the inflection point of ulna growth in tree
swallows. Tested are the effects of PLOT (test or control),
and nests (NEST) within a plot for 1985 through 1989.

YEAR	SOURCE	DF	TYPE III SS	MS	F	P > F
1989	PLOT	1	0.003	0.003	0.69	0.500
	NEST(PLOT)	14	0.058	0.004	2.86	0.005
	ERROR	38	0.055	0.001		
1988	PLOT	1	1.091	1.091	0.75	0.394
	NEST(PLOT)	28	40.781	1.456	4.66	0.0001
	ERROR	117	36.568	0.313		
1987	PLOT	1	3.213	3.213	1.76	0.197
	NEST(PLOT)	24	43.860	1.828	6.13	0.0001
	ERROR	80	23.841	0.298		
1986	PLOT	1	0.062	0.062	0.08	0.783
	NEST(PLOT)	12	9.281	0.773	5.12	0.0001
	ERROR	45	6.794	0.151		
1985	PLOT	1	6.516	6.516	1.61	0.217
	NEST(PLOT)	23	92.909	4.040	14.11	0.0001
	ERROR	80	22.909	0.286		

Table 32. Nested ANOVA for wing growth in tree swallows. Tested are the effects of PLOT (test or control), and nests (NEST) within a plot for 1985 through 1989.

YEAR	SOURCE	DF	TYPE III SS	MS	F	P > F
1989	PLOT	1	0.00003	0.00003	0.18	0.68
	NEST(PLOT)	14	0.002	0.00002	12.96	0.0001
	ERROR	38	0.0004	0.00001		
1988	PLOT	1	0.00009	0.00009	0.20	0.66
	NEST(PLOT)	28	0.012	0.0004	3.22	0.0001
	ERROR	118	0.015	0.0001		
1987	PLOT	1	0.00001	0.00001	0.04	0.851
	NEST(PLOT)	24	0.009	0.0004	4.81	0.0001
	ERROR	79	0.006	0.00008		
1986	PLOT	1	0.0003	0.0003	0.54	0.477
	NEST(PLOT)	12	0.007	0.0006	10.56	0.0001
	ERROR	45	0.002	0.00006		
1985	PLOT	1	0.002	0.002	1.60	0.218
	NEST(PLOT)	23	0.032	0.001	12.76	0.0001
	ERROR	80	0.009	0.0001		

Table 33. Tree swallow growth constants derived from fitted growth curves. Data are from test (Pirlot Road) and control (Tachycineta Meadows) sites for 1985 through 1989. N = number of nestlings [a]

Variable	Year	TEST				CONTROL			
		N	$\bar{X}$	SD	CV	N	$\bar{X}$	SD	CV
Weight	1989	24	0.48	0.08	17.00	30	0.49	0.08	16.90
	1988	72	0.38	0.09	23.71	70	0.35	0.09	25.81
	1987	44	0.40	0.10	25.18	62	0.39	0.10	24.54
	1986	19	0.42	0.06	15.05	41	0.42	0.06	15.15
	1985	80	0.44	0.11	24.47	29	0.44	0.08	18.84
Tarsus	1989	24	0.30	0.05	15.40	30	0.26	0.04	14.80
	1988	72	0.26	0.06	22.99	58	0.26	0.08	31.85
	1987	41	0.25	0.06	24.83	59	0.24	0.08	32.66
	1986	18	0.22	0.05	25.14	40	0.29	0.07	23.68
	1985	79	0.35	0.12	33.67	29	0.31	0.07	22.69
Ulna	1989	24	0.31	0.05	17.20	30	0.33	0.04	12.00
	1988	74	0.30	0.05	16.44	73	0.28	0.06	20.92
	1987	44	0.35	0.05	14.82	62	0.34	0.05	15.32
	1986	18	0.39	0.06	16.71	41	0.39	0.05	13.38
	1985	79	0.34	0.11	32.97	29	0.36	0.10	22.71
Wing	1989	24	0.16	0.01	5.50	30	0.16	0.01	3.00
	1988	74	0.16	0.01	7.08	74	0.16	0.02	10.02
	1987	43	0.16	0.01	7.78	62	0.16	0.01	7.22
	1986	18	0.16	0.01	5.92	41	0.17	0.01	8.28
	1985	80	0.18	0.02	12.45	29	0.17	0.01	7.48

[a]The numbers in this table are from completely reanalyzed data and may not agree with figures in earlier annual reports.

Table 34. Tree swallow inflection points derived from fitted growth curves. Data are from test (Pirlot Road) and control (Tachycineta Meadows) sites for 1985 through 1989. N = number of nestlings.[b]

Variable	Year	TEST				CONTROL			
		N	$\bar{X}$	SD	CV	N	$\bar{X}$	SD	CV
Weight	1989	24	5.48	1.08	19.80	30	5.83	0.77	13.20
	1988	72	5.54	0.94	17.05	70	5.40	0.84	15.51
	1987	44	5.83	0.89	15.21	62	5.58	0.96	17.27
	1986	19	6.08	0.75	12.30	41	6.15	0.72	11.66
	1985	80	5.20	0.80	15.40	29	5.79	1.02	17.55
Tarsus	1989	24	2.86	0.86	29.90	30	3.42	0.93	27.20
	1988	72	1.23	0.80	65.30	58	1.25	1.02	81.42
	1987	41	1.51	1.69	111.78	59	1.33	1.47	110.96
	1986	18	1.01	1.09	107.83	40	1.73	1.13	65.08
	1985	79	1.95	3.50	179.63	29	1.78	1.31	73.79
Ulna	1989	24	5.64	1.01	17.90	30	5.76	0.62	10.80
	1988	74	4.84	0.69	14.22	73	4.70	0.77	16.40
	1987	44	4.94	0.89	18.09	62	4.61	0.74	16.06
	1986	18	5.25	0.46	8.831	41	5.19	0.56	10.74
	1985	79	4.86	1.14	23.52	29	5.66	1.01	17.87

Wing [a]

[a] Inflection point not applicable to curves for wing growth.
[b] The numbers in this table are from completely reanalyzed data and may not agree with figures in earlier annual reports.

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Table 35. Minimum detectable differences and minimum percent detectable change in the mean to reach 70% certainty (power) of test for growth constants. [a] N = the number of nests per treatment for test or control. Detectable difference is in actual amount and percent of the grand mean.

Variable	Year	N	Actual Detectable Difference(%)	Actual Power	% Detectable Difference at 70% Power
Weight	1989	7	0.070(14.4)	<.30	0.190(39.0)
	1988	14	0.068(18.6)	<.30	0.130(35.6)
	1987	12	0.063(16.2)	<.30	0.165(42.3)
	1986	6	0.045(10.7)	<.30	0.103(31.0)
	1985	12	0.072(16.4)	<.30	0.188(42.7)
Tarsus	1989	7	0.09(30.9)	0.79	0.079(27.0)
	1988	13	0.018(6.9)	<.30	0.086(33.1)
	1987	12	0.041(17.1)	<.30	0.112(46.7)
	1986	6	0.083(30.7)	<.30	0.160(59.3)
	1985	12	0.053(15.6)	<.30	0.188(55.3)
Ulna	1989	7	0.020(5.3)	<.30	0.106(28.0)
	1988	14	0.012(4.1)	<.30	0.088(30.3)
	1987	12	0.020(5.9)	<.30	0.058(17.1)
	1986	6	0.036(9.2)	<.30	0.098(25.1)
	1985	12	0.052(14.9)	<.30	0.203(58.0)
Wing	1989	7	0.002(1.3)	<.30	0.006(4.0)
	1988	14	0.007(4.4)	<.30	0.020(12.4)
	1987	12	0.008(5.0)	<.30	0.021(13.1)
	1986	6	0.010(5.9)	<.30	0.038(22.4)
	1985	12	0.013(7.2)	<.30	0.034(18.9)

[a]The data in this table have been reanalyzed using N = number of nests per treatment and do not agree with figures in earlier annual reports.

Table 36. Minimum detectable differences and minimum percent detectable change in the mean to reach 70% certainty (power) of test for growth inflection point. * N = the number of nests per treatment for test or control.

Variable	Year	N	Actual Detectable Difference(%)	Actual Power	% Detectable Difference at 70% Power
Weight	1989	7	0.540(9.5)	<.30	2.05(36.0)
	1988	14	0.368(6.7)	<.30	1.43(26.1)
	1987	12	0.362(6.4)	<.30	1.65(29.0)
	1986	6	0.650(10.6)	<.30	1.75(28.5)
	1985	12	0.452(8.4)	<.30	1.50(28.0)
Tarsus	1989	7	0.640(20.0)	<.30	2.27(71.0)
	1988	13	0.404(32.6)	<.30	1.07(86.3)
	1987	12	0.965(68.9)	<.30	2.68(191.4)
	1986	6	0.592(39.2)	<.30	2.66(176.2)
	1985	12	0.574(30.2)	<.30	1.70(89.5)
Ulna	1989	7	0.020(0.3)	<.30	0.11(1.6)
	1988	14	0.228(4.8)	<.30	1.20(25.2)
	1987	12	0.480(10.1)	<.30	1.45(30.5)
	1986	6	0.487(9.3)	<.30	1.36(26.1)
	1985	12	0.642(12.7)	<.30	2.15(42.4)

Wing *

* Inflection point not applicable to curves for wing growth.
b The data in this table have been reanalyzed using N = number of nests per treatment and do not agree with figures in earlier annual reports.

Table 37. Statistics for growth rate of body mass for young deermice
compared by year and plot. Growth rate is the slope of a
linear model.

	Control				Test			
Year	N	$\bar{X}$	Std D	CV%	N	$\bar{X}$	Std D	CV%
1989	15	0.30	0.045	15.1	14	0.38	0.050	13.1
1988	32	0.33	0.062	18.6	35	0.37	0.064	17.1
1987	47	0.38	0.063	16.4	42	0.31	0.777	25.2
1986	42	0.25	0.091	36.2	50	0.28	0.085	29.9

Table 38. Nested ANOVA of deermice growth rates on test (Pirlot Road)
and control (Michigamme) sites for years 1989, 1988, 1987 and
1986. Tested are the effects of plots ,PLOT, and litters
within a plot, MOTHER(PLOT).

Year	Source	DF	Type III SS	MS	F-Value	P > F
1989	PLOT	1	0.058	0.058	4.60	0.097
	MOTHER(PLOT)	4	0.051	0.013	29.75	0.001
	ERROR	23	0.010	0.0004		
1988	PLOT	1	0.024	0.024	1.18	0.300
	MOTHER(PLOT)	11	0.221	0.020	28.81	0.0001
	ERROR	54	0.038	0.0007		
1987	PLOT	1	0.0008	0.0008	0.03	0.855
	MOTHER(PLOT)	14	0.338	0.0241	31.39	0.0001
	ERROR	71	0.055	0.0008		
1986	PLOT	1	0.054	0.054	3.21	0.095
	MOTHER(PLOT)	14	0.234	0.017	14.25	0.0001
	ERROR	70	0.082	0.001		

Table 39. Minimum detectable differences and power for deermice growth
constants for years 1986 - 1989.

Year	N	Actual Detectable Difference(%)	Actual Power	% Detectable Difference at 70% Power
1989	2	0.212(62.4)	.33	0.34(100.0)
1988	5	0.040(11.4)	<.30	0.24(68.6)
1987	7	0.262(74.9)	<.30	0.70(200.0)
1986	7	0.103(38.1)	.30	0.19(68.5)

Table 40. Relevant statistics for age of eye-opening and incisor
eruption for deermice reared in enclosures from 1985 through
1989.

Year	Plot	Eye Opening			Incisor Eruption		
		n	$\bar{X}$	SD	n	$\bar{X}$	SD
1989	Test	14	15.3	1.14	14	5.0	0.88
	Control	15	15.7	3.01	15	6.0	1.51
1988	Test	35	14.7	1.44	35	4.4	0.91
	Control	32	16.3	1.37	17	5.0	0.87
1987	Test	44	15.7	1.87	44	6.1	1.55
	Control	43	16.0	1.10	43	6.4	1.66
1986	Test	28	14.1	2.01	28	5.6	1.29
	Control	48	15.0	1.08	48	6.1	1.49

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Table 41. Nested ANOVA of deermice age of eye opening on test (Pirlot Road) and control (Michigamme) sites for years 1985 through 1989. Tested are the effects of plots, PLOT, and litters within a plot, MOTHER(PLOT).

Year	Source	DF	Type III SS	MS	F-Value	P > F
1989	PLOT	1	4.298	4.298	0.13	0.733
	MOTHER(PLOT)	4	128.457	32.114	48.17	0.0001
	ERROR	23	15.333	0.667		
1988	PLOT	1	37.020	37.020	3.92	0.073
	MOTHER(PLOT)	11	103.954	9.450	20.25	0.0001
	ERROR	54	25.200	0.467		
1987	PLOT	1	3.458	3.458	0.28	0.603
	MOTHER(PLOT)	14	170.739	12.196	28.74	0.0001
	ERROR	71	30.124	0.424		
1986	PLOT	1	9.630	9.630	0.76	0.400
	MOTHER(PLOT)	12	151.674	12.640	61.54	0.0001
	ERROR	62	12.733	0.205		

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Table 42. Nested ANOVA of deermice incisor eruption on test (Pirlot Road) and control (Michigamme) sites for years 1986 through 1989. Tested are the effects of plots, PLOT, and litters within a plot, MOTHER(PLOT).

Year	Source	DF	Type III SS	MS	F-Value	P > F
1989	PLOT	1	5.337	5.337	0.55	0.499
	MOTHER(PLOT)	4	38.800	9.700	69.72	0.0001
	ERROR	23	3.200	0.139		
1988	PLOT	1	3.703	3.703	0.73	0.417
	MOTHER(PLOT)	8	40.400	5.050	99999.99	0.000
	ERROR	42	0.000*	0.000		
1987	PLOT	1	5.650	5.650	0.40	0.535
	MOTHER(PLOT)	14	195.684	13.977	42.43	0.0001
	ERROR	71	23.391	0.329		
1986	PLOT	1	1.289	1.289	0.14	0.713
	MOTHER(PLOT)	12	109.279	9.107	13.98	0.0001
	ERROR	62	40.390	0.651		

* - very small number

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Table 43. Minimum detectable differences and power for deermice
maturation events for years 1986 through 1989.

Variable	Year	N	Actual Detectable Difference(%)	Actual Power	Detectable Difference at 70% Power(%)
Eye	1989	2	5.27(34.0)	<.30	16.8(108.4)
opening	1988	5	3.32(21.4)	.33	5.4(34.5)
days (%)	1987	7	1.58(10.0)	<.30	5.0(31.6)
	1986	6	1.00(6.8)	<.30	5.6(38.1)
Incisor	1989	2	2.09(38.0)	<.30	9.2(167.3)
eruption	1988	4	.82(17.8)	<.30	4.5(97.8)
days(%)	1987	7	1.54(24.9)	<.30	5.3(85.5)
	1986	6	1.61(27.4)	<.30	4.7(79.7)

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Table 44. Numbers of birds used in the tree swallow homing study and
likelihood to return following displacement, 1986-1988.
Returns are those birds which returned to the plot in less
than 300 minutes. Likelihood to return was assessed using
the Chi-squared statistic.

Year	Treatment	Return	Not Return	
1989	Test	14	0	Not tested- see text
	Control	15	1	
1988	Test	37	4	
	Control	39	6	$X^2 = 0.27$ $P > 0.5$
1987	Test	36	1	
	Control	25	13	$X^2 = 12.259$ $P < 0.001$
1986	Test	26	3	
	Control	24	7	$X^2 = 1.6$ $P > 0.1$

Table 45. Mean return times of tree swallows in minutes for 1986-1989 field seasons. Data for the two test sites were pooled after determining that there were no significant differences between them.

Year	Treatment	X	SD	n
1989	Test	118.1	47.7	14
	Control	168.1	53.4	15
1988	Test	136.8	39.5	37
	Control	189.8	54.4	39
1987	Test	155.1	46.2	36
	Control	202.4	55.6	25
1986	Test	149.8	52.6	26
	Control	176.9	67.0	22

Table 46. Analysis of variance comparing tree swallow return times in
minutes for 1986-1988 field seasons. Variables considered
are PLOT(test and control), YEAR and PLOT/YEAR interaction.

Source	DF	Type III SS	MS	F	P > F
PLOT	1	92039.893	92039.893	34.46	0.0001
YEAR	3	25364.368	8454.789	3.17	0.0255
PLOT*YEAR	3	5320.003	1773.334	0.66	0.5751
ERROR	206	550200.102			

Table 47. Data on tree swallow likelihood to return pooled over all
years (1986-1989) for test and control plots.

Plot	Return	Not Return	
Test	113	8	
Control	103	27	$X^2 = 10.873$ $P < 0.001$

Table 48. Detectable differences and power for tree swallow homing:
return time for years 1986 - 1989. N = number of adults per
treatment for test or control. Differences presented in
minutes with % following.

Variable	N	Actual Detectable Difference(%)	Actual Power	Detectable Difference (%) at 70% Power
Return time minutes(%)	103	41.66(25.4)	>.99	18.0(11.0)

Table 49. Results of the small mammal homing studies at Pirlot Road
test site and Michigamme control site during the summer of
1988.

Species	Year	Plot	Return	Not Return	
Chipmunks	1989	Test	15	8	G=0.31
		Control	9	7	P>0.50
	1988	Test	5	12	
		Control	2	3	
	1987	Test	4	8	
		Control	2	2	
	1986	Test	13	6	
		Control	20	2	
			Overall G=21.299	P<0.005	
Deermice	1989	Test	13	8	G=4.830
		Control	3	10	P<0.05
	1988	Test	17	24	
		Control	9	5	
	1987	Test	16	7	
		Control	6	3	
	1986	Test	5	1	
		Control	1	2	
			Overall G=13.795	NS	

Table 50. Frequency of tree swallow abnormalities. First number is the number of embryos displaying abnormality. Second number (in parentheses) is the number of nests involved.

	No Development	Chaotic	Rotation Reversed	Spine Abnormal	Allantois Reversed
Test	2 (2)	1 (1)	1 (1)	7 (4)	2 (2)
Control	1 (1)	0	0	6 (3)	2 (2)
Percentage Abnormal	1.9%	0.6%	0.6%	8.2%	2.5%

Table 51. Chi-square analysis of developmental abnormalities found in early tree swallow embryos collected from test and control sites. Analysis excludes embryos with posteriorly directed allantois, since these are probably a variation of normal development.

	Test	Control	Total
Normal	67	72	139
Abnormal	11	8	19
Total	78	80	158

$X^2 = 0.63$
Contingency coefficient = 0.06

Table 52. Chi-square analysis of development abnormalities found in early tree swallow embryos collected from test and control sites. Analysis includes embryos with posteriorly directed allantois, which were excluded from Table 51.

	Test	Control	Total
Normal	65	71	136
Abnormal	13	9	22
Totals	78	80	158

$X^2 = 0.97$
Contingency coefficient = 0.08

Table 53. Chi-square analysis of development abnormalities found in early tree swallow embryos collected from test and control sites. Analysis excludes both embryos with dorsally-directed allantois and "dented" backs.

	Test	Control	Totals
Normal	71	76	147
Abnormal	7	4	11
Total	78	80	158

$X^2 = 0.96$
Contingency coefficient = 0.08

Table 54. Calculated volumes of tree swallow eggs collected from test and control plot.

Test				Control		
Nest No.	Mean Volume (cc)	S.D.		Nest No.	Mean Volume (cc)	S.D.
1	1.76	0.03		5	1.73	0.09
2	1.86	0.14		6	1.65	0.06
3	1.75	0.05		7	1.90	0.07
4	1.74	0.11		11	2.02	0.05
8	1.66	0.07		12	1.80	0.04
9	1.73	0.06		13	1.51	0.06
10	1.54	0.06		15	1.80	0.09
14	1.94	0.09		16	1.77	0.03
19	1.92	0.04		17	1.60	0.04
20	1.58	0.04		18	1.87	0.06
21	1.75	0.08		26	1.77	0.05
22	1.68	0.06		27	1.76	0.08
23	1.79	0.07		28	1.81	0.14
24	1.93	0.05		29	1.60	0.04
25	1.87	0.06		30	1.80	0.07

Table 55. Nested Analysis of Variance of egg volume for test and control plots and eggs within nests. Means ± Standard Deviation are shown for each plot.

Source	DF	MS	F	P
PLOT (Test $\bar{X}=1.77\pm0.13$) (Control $\bar{X}=1.76\pm0.14$)	1	0.0022	0.03	>0.87
EGG(NEST)	28	0.084	16.90	<0.0001
ERROR	130	0.647		

Table 56. Summary of peak metabolic rates measured on deermice and
chickadees in the week following capture in 1989. All
measured peaks, regardless of their quality rating, are
reported for the deermice. For the chickadees, both all
measured peaks and the peaks in quality classes 1-4 are
summarized.

Species and Plot	Number of Measures	Peak Metabolic Rate [ml O_2/(g · hr)] Mean	S.D.	Mean Body Weight (g)
Deermice				
MGE (Control)	10	19.4	1.1	18.6
PRT (Test)	7	18.5	1.3	19.5
Chickadees -- all measured peaks				
MGE	12	25.1	1.8	10.9
PRT	12	24.1	2.2	10.9
Chickadees -- peaks in classes 1-4				
MGE	10	25.2	1.9	10.9
PRT	8	24.8	2.1	10.9

Table 57. Mean and standard deviation of weight-specific peak metabolic
rate pooled across all plots and years, plus estimated sample
sizes required to be able to detect a 20% difference between
test and control plots at the 5% level of significance with
90% certainty. Sample size estimates were made by the
procedure of Sokal & Rohlf (1981, p. 263).

Species	Number of Measures	Peak Weight-Specific Metabolic Rate $[ml\ O_2/(g \cdot hr)]$		Estimated Minimal Per-Plot Sample Size Required to Meet Standard of Statistical Sufficiency
		Mean	S.D.	
Deermouse	79	19.73	1.85	6
Chickadee				
Primary-quality measures only	78	24.76	1.87	5
All measures	112	24.37	1.90	5

Table 58. Initial determination of peak metabolic rate in two
individuals. The peak rates are underlined.

Species	Number of Days Since Capture	Ambient Temperature (°C)	Body Temperature (°C)		Oxygen Consumption in Helium-Oxygen (ml/hr)
			Start	End	
Deermouse	4	0.8	36.8	35.3	313.8
(22.3 g)	2	-4.8	38.4	33.7	348.9
	3	-8.4	35.9	32.3	343.2
Chickadee	5	0.0	42.5	42.1	253.6
(10.5 g)	1	-3.6	42.7	39.5	281.9
	3	-9.6	43.2	38.0	264.1

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Table 59. The initial peak metabolic rate and the metabolic rates at retesting in the two individuals shown in Table 58.

Species	Number of Days since Capture	Ambient Temperature (°C)	Body Temperature (°C)		Oxygen Consumption in Helium-Oxygen (ml/hr)
			Start	End	
. Deermouse	2	-4.8	38.4	33.7	348.9
	12	-4.1	37.0	37.1	343.0
	17	-4.9	35.8	35.1	348.8
Chickadee	1	-3.6	42.7	39.5	281.9
	11	-4.2	42.8	40.5	286.7
	18	-4.9	43.5	40.3	260.7

Table 60. Ambient temperatures at which peak metabolic rates occurred.

Nominal Temperature (°C)	Temperature Range (°C)	Number of Animals Exhibiting Peak in Specified Range	
		Deermice	Chickadees
5	2.5 to 7.4	1	1
0	-2.5 to 2.4	0	2
-5	-7.5 to -2.6	8	14
-10	-12.5 to -7.6	15	12
-15	-17.5 to -12.6	10	3
-20	-22.5 to -17.6	6	0

Table 61. Initial peak metabolic rates as a function of the time in captivity when the peak rates were measured.

Number of Days in Captivity	Number of Animals Exhibiting Peak on Given Day	Weight-Specific Peak Rate of Oxygen Consumption of Those Animals (ml O_2/g·h)		Final Body Temperatures of Those Animals (°C)	
		Mean	S.D.	Mean	S.D.
Deermice					
1	8	20.2	1.5	34.5	2.0
2	8	18.8	2.3	34.5	1.6
3	8	19.4	2.2	34.1	2.6
4	7	19.0	1.7	31.6	3.0
5	5	20.5	1.6	32.1	1.8
6	1	21.4	---	33.2	---
8	3	18.9	2.0	27.6	3.1
Chickadees					
1	13	24.0	1.6	39.8	1.9
2	5	24.3	1.6	40.0	1.7
3	5	24.8	1.3	38.6	1.2
4	4	24.5	2.2	40.1	0.5
5	3	25.1	2.2	38.1	5.4
8	2	26.0	4.8	38.4	2.2

Table 62. Total number of runs employed in determination of initial peak metabolic rate.

Number of Runs	Number of Individuals Exposed to Specified Number of Runs	
	Deermice	Chickadees
3	25	29
4	14	2
5	1	1

Table 63. Initial peak metabolic rate as a function of the number of
runs preceding the peak run itself.

Number of Runs Before Peak Run	Number of Animals Exhibiting Peak After Given Number of Runs	Weight-Specific Peak Rate of Oxygen Consumption of Those Animals $(ml\ O_2/g \cdot h)$	
		Mean	S.D.
Deermice			
0	12	19.5	1.8
1	12	19.4	1.8
2	11	19.4	2.1
3	5	20.2	2.3
Chickadees			
0	15	24.0	1.6
1	11	24.8	1.4
2	4	24.5	2.2
3	2	26.0	4.8

Table 64. Times between capture, initial peak determination, and retesting for the early-retest and late-retest sets of both species.

| | Number of Individuals Exhibiting Specified Delay | | | |
| | Deermice | | Chickadees | |
Number of Days	Capture to Peak Determination	Capture to Retesting	Capture to Peak Determination	Capture to Retesting
Early-Retest Groups				
1	6		13	
2	8		4	
3	7		5	
4	7		4	
5	4		2	
6	1		0	
9		0		2
10		0		2
11		3		7
12		·22		12
13		4		4
14		4		1
Late-Retest Groups				
1	4		10	
2	6		5	
3	6		3	
4	4		3	
5	3		2	
6	1		0	
16		0		4
17		3		0
18		11		14
19		9		2
20		0		2
21		0		0
22		1		1

Table 65. Initial peak metabolic rates and metabolic rates at retesting in the early- and late-retest sets of both species. The differences between retest values and initial peaks were calculated individual by individual.

| | Oxygen Consumption (ml/hr) | | | | | |
| | Initial Peak | | Retest | | Retest Value Minus Initial Peak | |
Set	Mean	S.D.	Mean	S.D.	Mean	S.D.
Deermice Early-Retest	360.6	32.0	372.4	37.0	11.8	26.9
Deermice Late-Retest	369.0	39.9	372.2	47.2	3.2	35.9
Chickadee Early-Retest	282.2	20.0	275.6	23.7	-6.6	27.0
Chickadee Late-Retest	284.2	23.0	287.3	20.3	3.2	30.0

Table 66. The special set of retest data. Each line refers to a different individual. Only the first individual contributed data to the early- and late-retest sets. The others contributed only to this set.

Species	Number of Days After Capture When Metabolic Rate Was Determined		Oxygen Consumption (ml/hr)	
	Initial Peak	Retest	Initial Peak	Retest
Deermouse	6	29	351.2	368.0
Deermouse	8	13	349.6	385.6
Deermouse	8	13	367.0	388.4
Deermouse	8	13	377.9	380.9
Chickadee	8	16	252.8	278.2
Chickadee	8	18	355.4	292.7

Table 67. Body weights and final body temperatures in the early- and
late-retest sets.

	Body Weight (g)				Final Body Temperature (°C)			
	At Time of Initial Peak		At Time of Retest		At Time of Initial Peak		At Time of Retest	
Set	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Deermice Early-Retest	18.5	2.7	19.1	2.7	33.4	2.5	34.6	2.5
Deermice Late-Retest	19.3	3.2	19.8	2.9	34.0	2.3	34.5	3.1
Chickadee Early-Retest	11.6	0.59	11.8	0.64	39.4	2.2	38.6	3.0
Chickadee Late-Retest	11.7	0.51	11.9	0.72	39.4	2.2	38.4	2.2

Table 68. Summary of major findings by task for 1985-1989.

Task	Results	Year
SMALL MAMMAL COMMUNITIES[a]		
Species richness.	.Test greater than Control. .	88,87
Species composition	.No Plot effect	88,87,86,85
Species diversity	.Test greater than Control. .	88
	Test less than Control . . .	87
	No Plot effect	86,85
Evenness.	.Test greater than Control. .	88
	Test less than control . . .	87
	No Plot effect	86,85
TPN - chipmunk	.Test less than Control . . .	88,87,86,85
TPN - deermouse.	.Test greater than Control. .	88,87
	No Plot effect	86,85
TREE SWALLOW - FECUNDITY, GROWTH AND MATURATION STUDIES		
Mean clutch size.	.No Plot or Year effect . . .	all years
Distrib. of clutch size. .	No Plot or Year effect . . .	all years
Likelihood to hatch	.No Plot or Year effect . . .	all years
Hatch rate.	.Year effect, no Plot effect.	all years
Likelihood to fledge. . . .	.Year effect, no Plot effect.	all years
Number fledged.	.Year effect, no Plot effect.	all years
Landmark growth events ·		
Eye opening	.Nest effect, no Plot effect.	89,88,87,86
Feather eruption.	.Nest effect, no Plot effect.	89,88,87,86
Mortality		
Egg	.Test greater than Control. .	87
	Test less than Control . . .	88,86
	No Plot effect	89,85
Nestling.	.Test greater than Control. .	86
	Test less than Control . . .	85,88
	No Plot effect	89,87
Overall Nest.	.Test greater than Control. .	88,87
	Test less than Control . . .	85
	No Plot effect	89,86
Incubation Phase/Nest . .	.Test greater than Control. .	87
	Test less than Control . . .	85
	No Plot effect	89,88,86
Nestling Phase/Nest . . .	.Test greater than Control. .	88
	No Plot effect	89,87,86,85

Table 68. (Continued) Summary of major findings by task for 1985-1989.

Task	Results	Year
TREE SWALLOW - FECUNDITY, GROWTH AND MATURATION STUDIES		
Tree Swallow Growth		
Weight increase	Nest effect, No Plot effect.	89,88,87,86,85
Weight inflection pt.	Nest effect, No Plot effect.	89,88,87,86,85
Tarsus growth	Nest and Plot effect	89
	No Nest or Plot effect	88
	Nest effect, No Plot effect.	87,86,85
Tarsus inflection pt.	No Nest effect, No Plot effect	88
	Nest effect, No Plot effect.	89,87,86,85
Ulna growth	Nest effect, No Plot effect.	89,88,85
	No Nest effect, No Plot effect	87,86
Ulna inflection pt.	Nest effect, No Plot effect.	89,88,87,86,85
Wing growth	Nest effect, No Plot effect.	89,88,87,86,85
Incubation[a]	Nest effect, No Plot effect, No Year effect	
	Ambient Temperature effect	88,87
DEERMOUSE - PARENTAL CARE, FECUNDITY, GROWTH AND MATURATION STUDIES		
Deermouse Growth		
Growth rate	Mother effect, No Plot effect.	89,88,87,86
Eye opening	Mother effect, No Plot effect.	89,88,87,86
Incisor eruption.	Mother effect, No Plot effect.	89,88,87,86
TREE SWALLOW HOMING STUDIES (Pooled data for 1986-1989)		
Likelihood to Return.	Test greater than Control.	86-89[b]
Mean Return Times	Test less than Control	86-89
SMALL MAMMAL HOMING STUDIES		
Likelihood to Return		
Chipmunk.	No Plot effect	89,88,87
Deermouse	No Plot effect	88,87
	Test greater than Control.	89
DEVELOPMENTAL STUDIES		
Abnormality Frequency	No Plot effect	all years
Egg Volume.	Nest effect, no Plot effect.	89
MAXIMUM AEROBIC METABOLISM STUDIES		
Peak Metabolic Rates		
Deermouse	No Plot or Year effect	all years
Chickadee	Test less than Control	88,87,86
	No Plot effect	89

[a] Study element dropped in 1989 due to budget constraints. See text.
[b] Not tested in 1989 due to small sample N caused by inclement weather. Pooled over years including 1989, test plots have greater return rates than control.

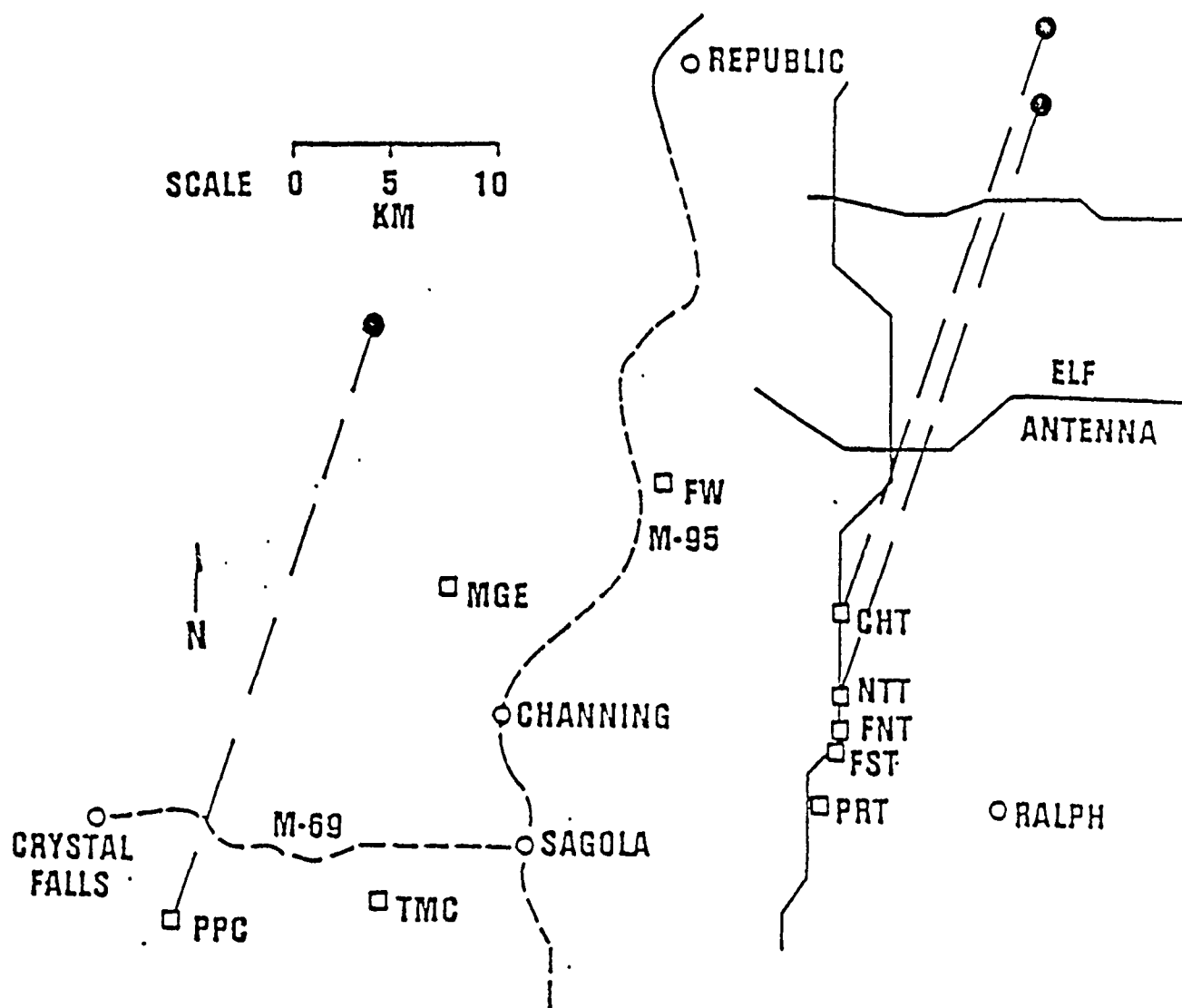Figure 1. Location of Test and Control plots in relation to antenna
system. See Table 1 for translation of plot codes.
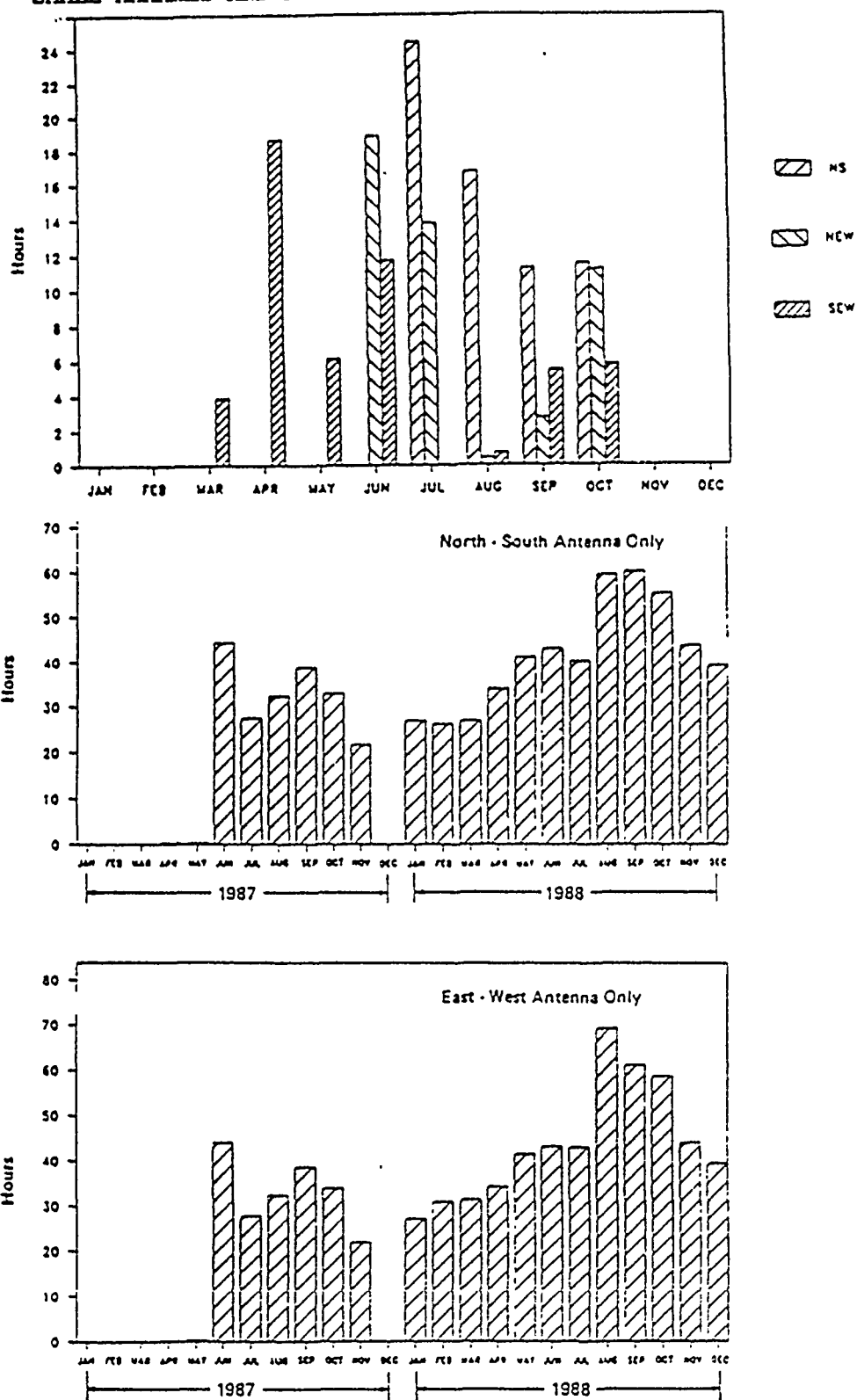
Figure 2. Operation schedule for antenna segments in 1986, 1987 and 1988. Data from IITRI (1989).
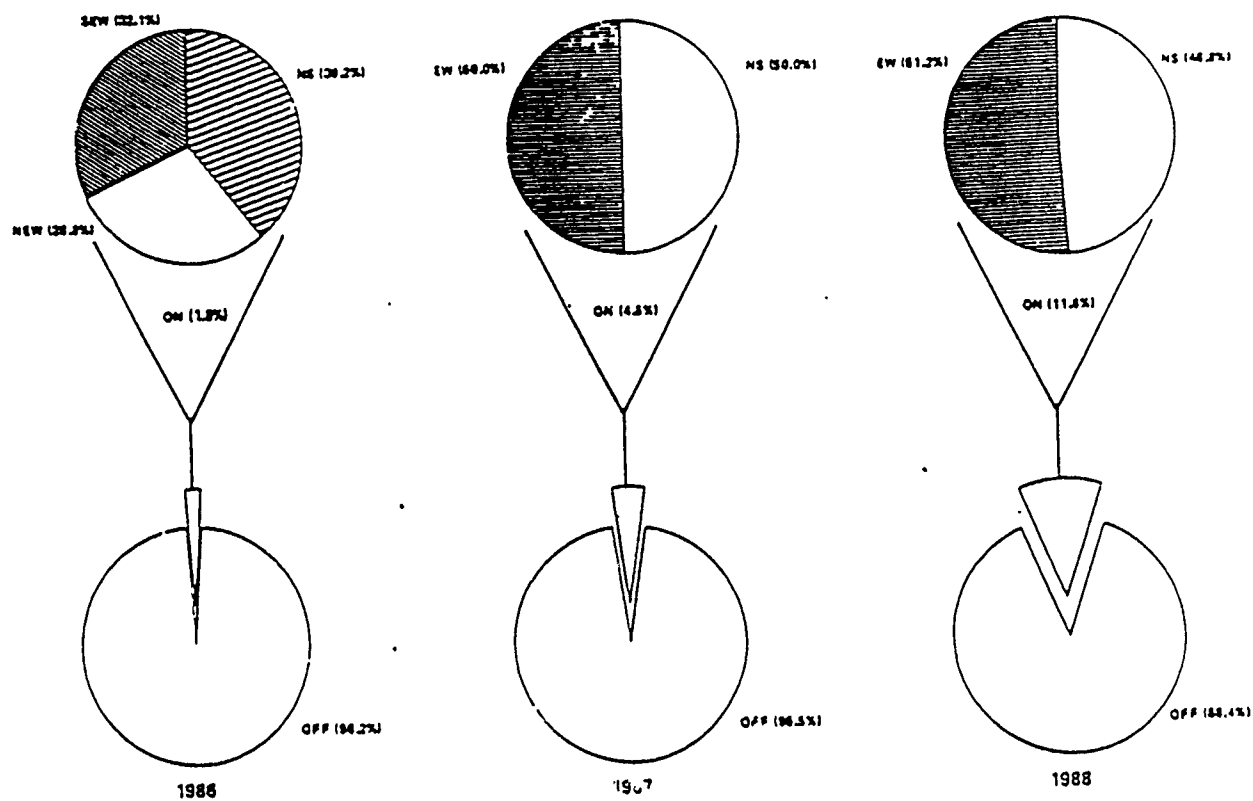
Figure 3. Percentage of operation by year, 1986-1988. Data from IITRI (1989).
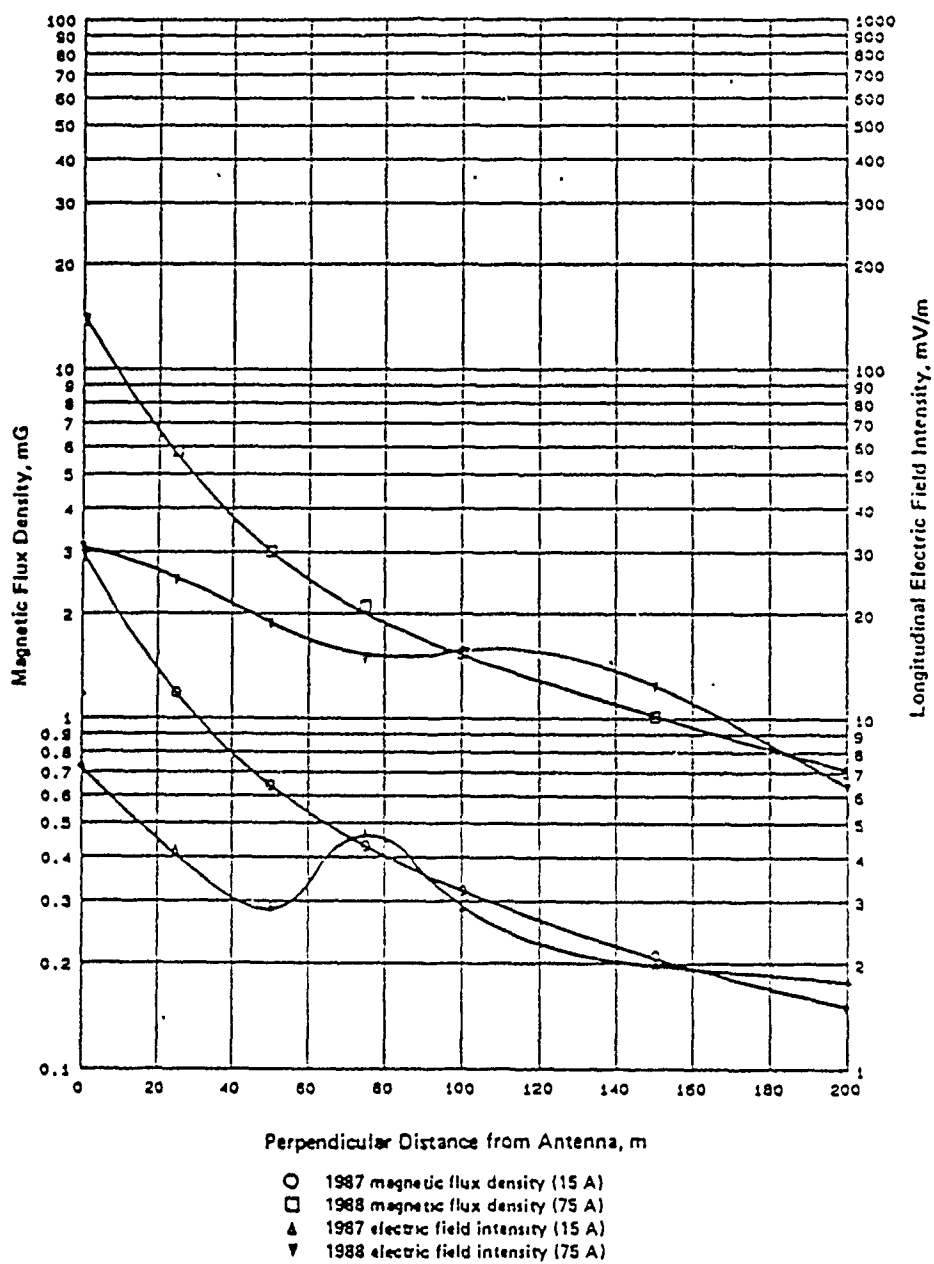
Figure 4. Profile of field strengths on test plot by year, 1987, 1988.
Data from IITRI (1989).
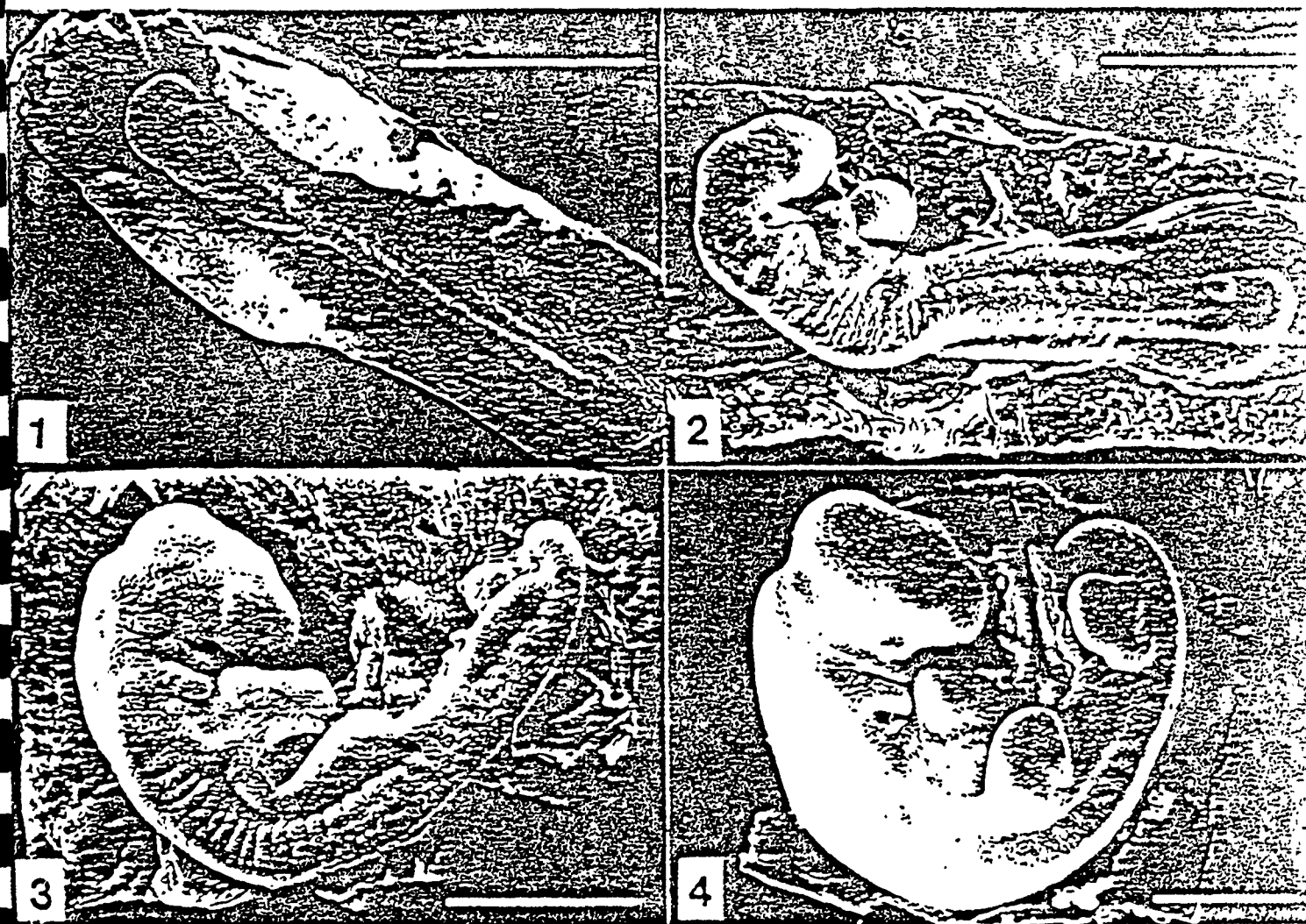
Plate 1. Normal tree swallow embryos (scanning electron microscopy).
1. Stage 10. 2. Stage 14. 3. Stage 20. 4. Stage 24. (Stages
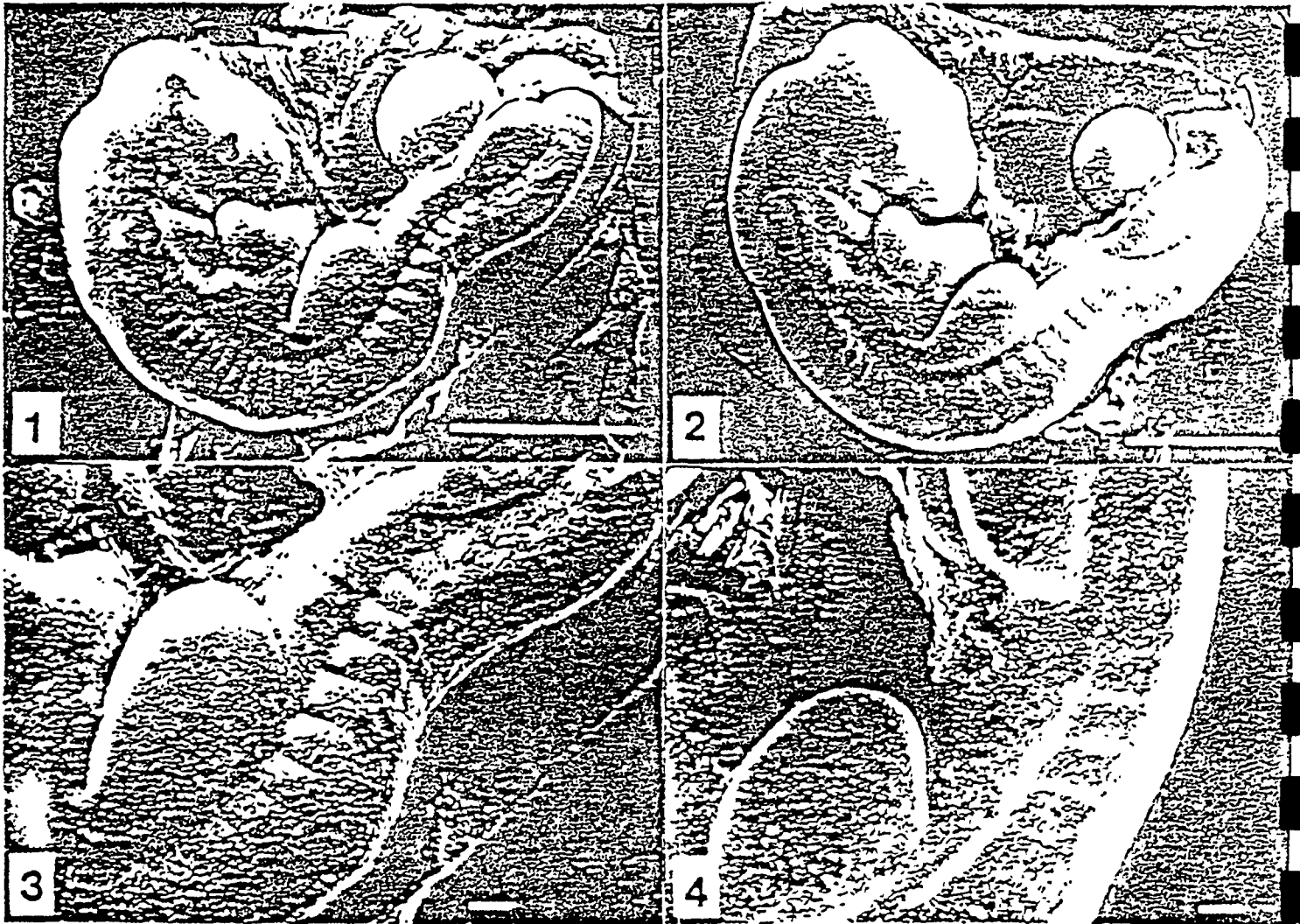are Hamilton - Hamburger scale)

Plate 2. Tree swallow embryos showing "dented" backs. 1 and 2.
          sibling embryos. 3. same embryo as 1 at higher magnification.
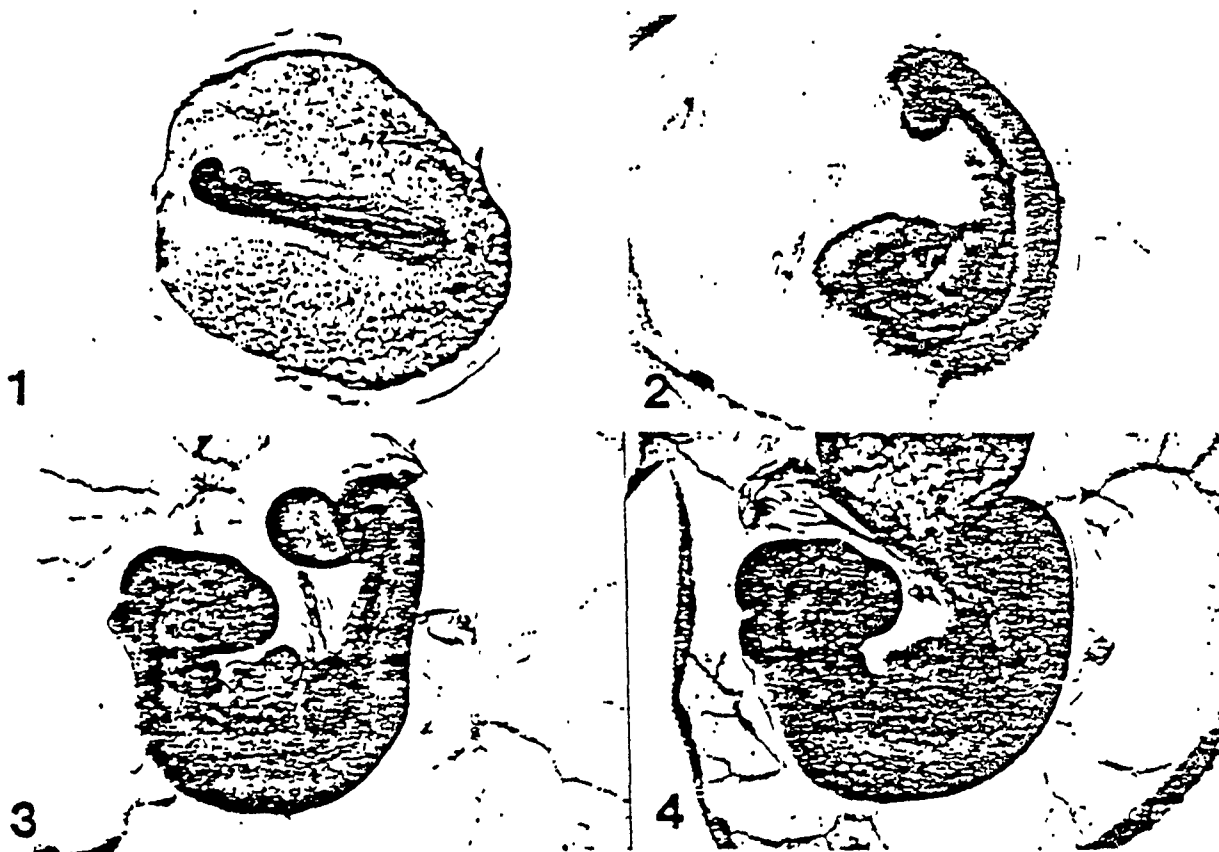   4. Normal back.

Plate 3. Normal tree swallow embryos, whole mount. 1. Stage 14. 2. Stage 20. 3. Stage 23. 4. Stage 25. (Stages are Hamilton - Hamburger scale)
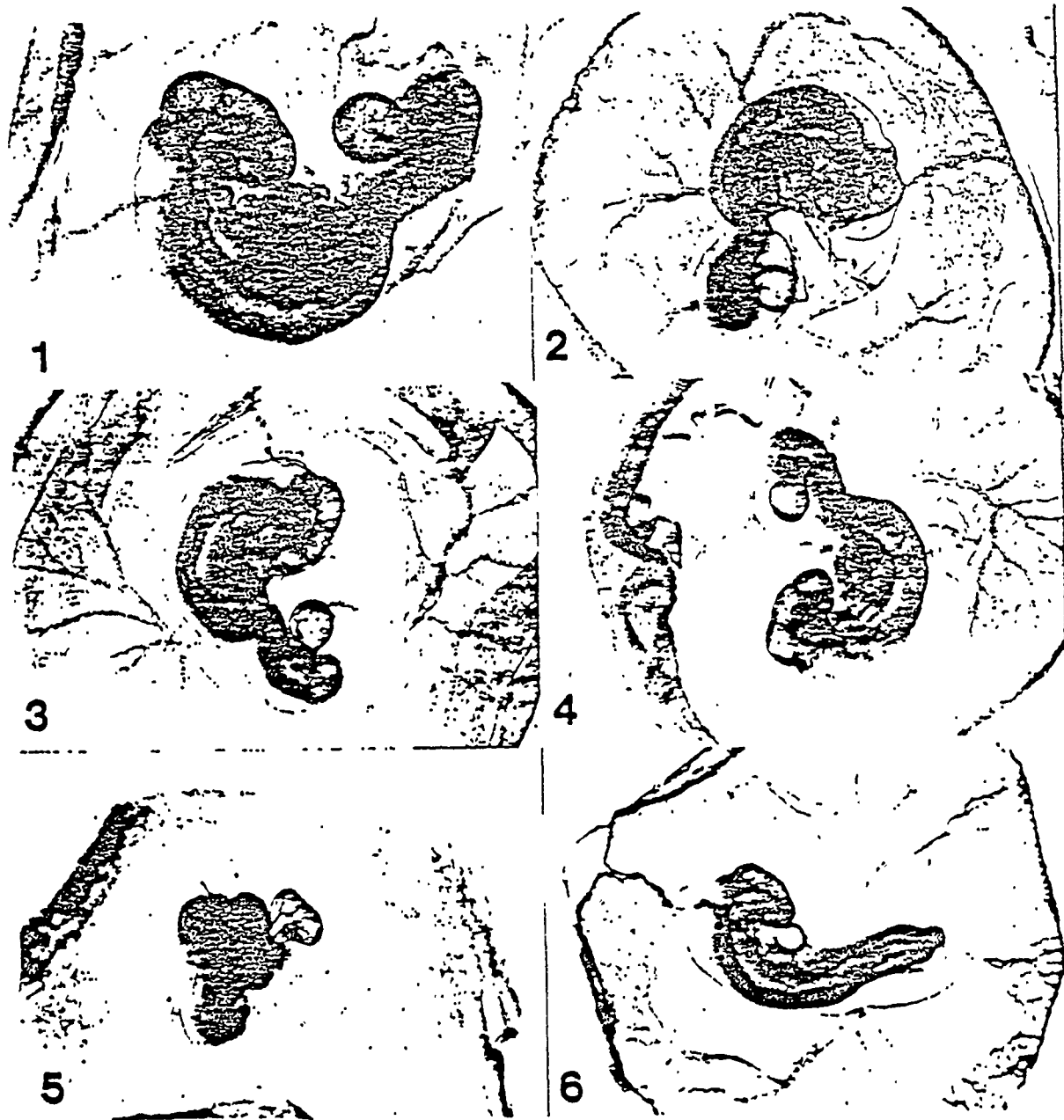
Plate 4. Abnormal tree swallow embryos. 1 - 4. Siblings with "dented" backs. 5. Abnormal embryo in which only the ventricle is clearly recognizable. 6. Embryo in which the allantois appears to be growing posteriorly creating the appearance of a bifurcated tail.